# THE ROYAL COLLEGE OF PATHOLOGISTS

## Part 2 Examination

## Monday 9 May 2005

## MEDICAL MICROBIOLOGY

## First Paper

**Candidates must answer either Question 1 or 2 in Section A and three questions from Section B**

*Time allowed - THREE HOURS*

### Section A

Please answer **either** Question 1 **or** Question 2:

1. Describe the epidemiological and clinical characteristics of sexually transmitted *Chlamydia trachomatis* infection. Describe the factors that should be considered in establishing a screening programme for this infection, and the diagnostic options available.

2. Your district general hospital has several endoscopy units on site with different procedures for decontamination. You have therefore been asked to review and standardise endoscopy decontamination services. Give recommendations based on best practice guidelines.

**Please turn over for Section B**

**Section B**

Please answer three of the following questions:

3. Write short notes on three of the following:

   a) ESBL producing *Escherichia coli*
   b) Panton-Valentine leucocidin positive MRSA
   c) Vancomycin-intermediate *Staphylococcus aureus*
   d) Multiple Drug resistant *Acinetobacter baumanii*

4. Write short notes on three of the following:

   a) linezolid
   b) Voriconazole
   c) non-nucleoside reverse transcriptase inhibitors
   d) Liposomal amphotericin

5. Write short notes on three of the following:

   a) Metapneumovirus
   b) Avian influenza
   c) Parvovirus infection
   d) Oseltamavir

6. Write short notes on three of the following:

   a) S. pyogenes pathogenesis
   b) Trophyrema whipplei
   c) *Neisseria meningitides* vaccines
   d) Pneumococcal conjugate vaccines

# THE ROYAL COLLEGE OF PATHOLOGISTS

**Part 2 Examination**

**Monday 9 May 2005**

**MEDICAL MICROBIOLOGY**

**Second Paper**

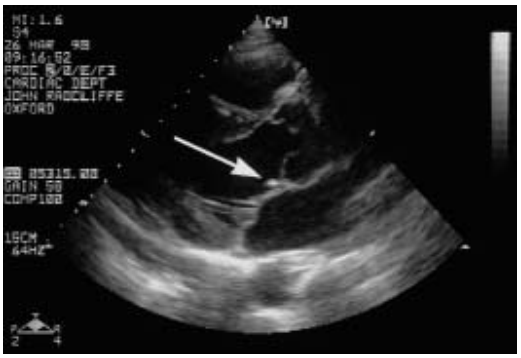
**Candidates must answer all questions in Section A (short answer questions) and two out of three questions in Section B (journal article evaluation questions)**

*Time allowed - THREE HOURS*

## Section A - Short Answer Questions

*Answer all questions.*
*The answers will usually be a single word or a few words.*
*There are no negative marks.*
*When the number of responses is specified* ***DO NOT give additional responses*** *as these will* ***NOT be marked.***

1. A 46 year old man who had been previously well presented with a history of fever and weight loss of 4 kg over the past months. His Hb was 8.9g/dL mcv 75.2 and weight loss. Three sets of blood cultures taken 3 weeks ago in the medical admission unit have not grown an organism. He has not been given any antibiotics. Below is illustrated a transthoracic echo-cardiogram.

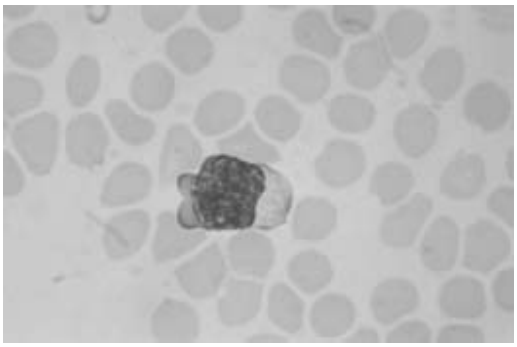



a) What is the likely diagnosis?

b) List three organisms are likely the causes of culture negative endocarditis?

c) What treatment would you advise?

2. This 18 year old female student presented with fever, sore throat and was treated with amoxicillin by her GP. She developed a rash. The biomedical scientist alerts you to her blood film which is illustrated below.

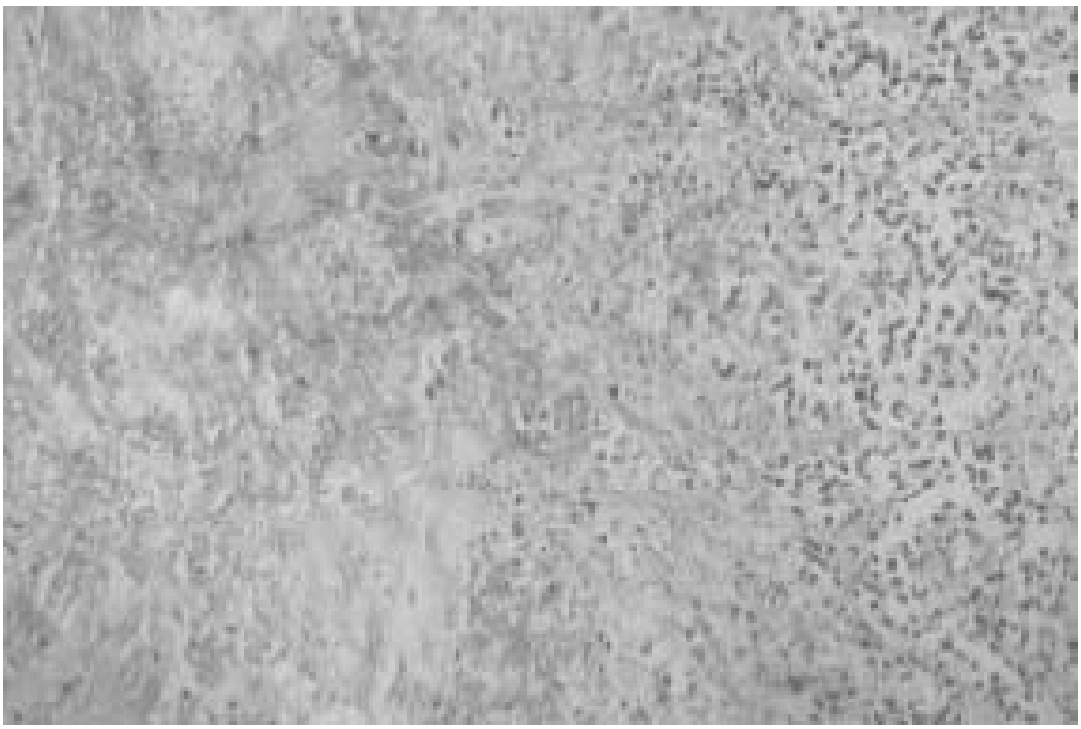


a) What is the likely diagnosis?

b) What test would you recommend to clinch the diagnosis?

c) What complications would you advise the clinicians to anticipate?

3.



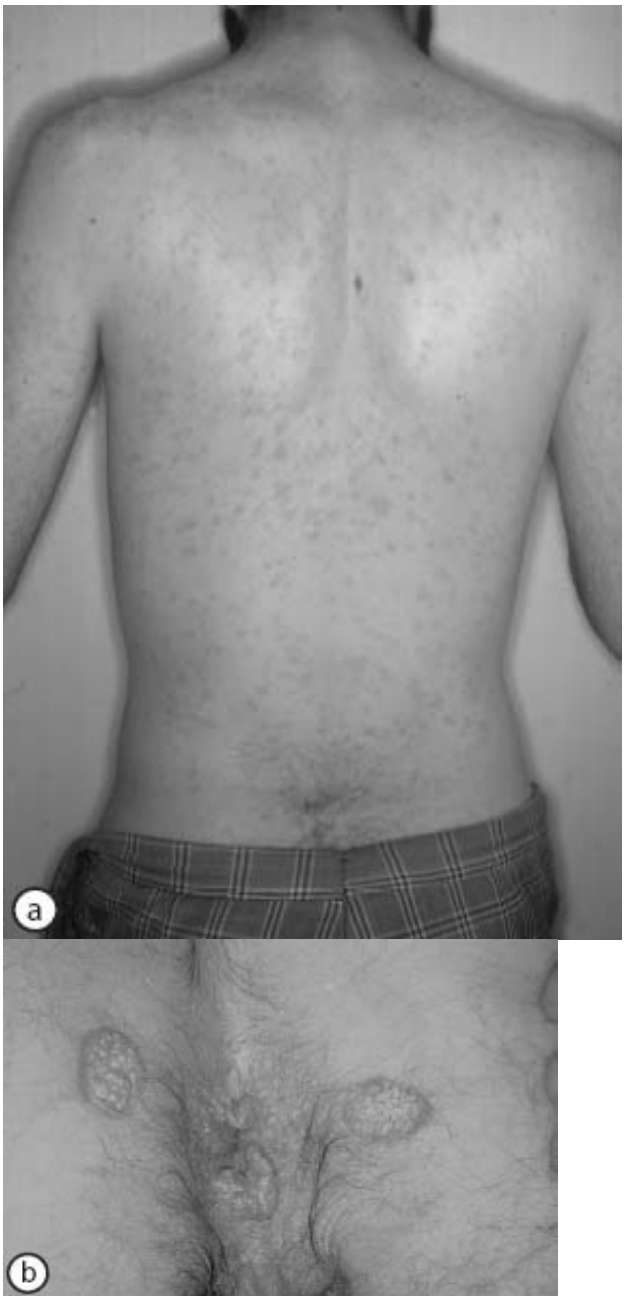
This is a haematoxylin eosin stained section of a lung biopsy of a febrile renal transplant patient with multiple infiltrates on chest xray.

a) What is the likely pathogen?

b) What are important risk factors for getting this infection? (give 2)

c) How do you treat this infection? (give 2 drug regimens)

4. This 25 year old male patient presented to casualty with this rash and these lesions on the perineum. You are asked for your opinion.

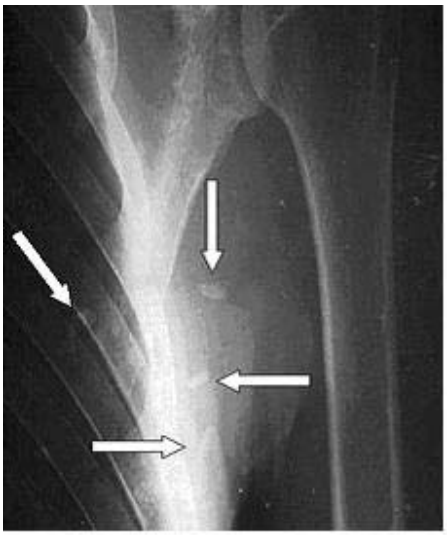



a) What is this infection?

b) Which microbiological tests should be done to confirm this infection? (give 2 )

c) How would you suggest this patient be managed?

5 A Peruvian pan-pipe player has an epileptic fit whilst playing in an underground station. He has never had a seizure before. During the course of his investigations he has a chest X ray that shows the following:



a) Give one site where these lesions are these lesions classically found in large quantities? (give one site)

b) What is the likely pathological diagnosis and causative organism?

c) What investigations (give one) should be performed and treatment given to this patient?

6. This is a summary of the findings on lumbar puncture of a 50 year old builder from Somerset who has gradual onset of headache, photophobia, low grade fever and no neurological deficits.

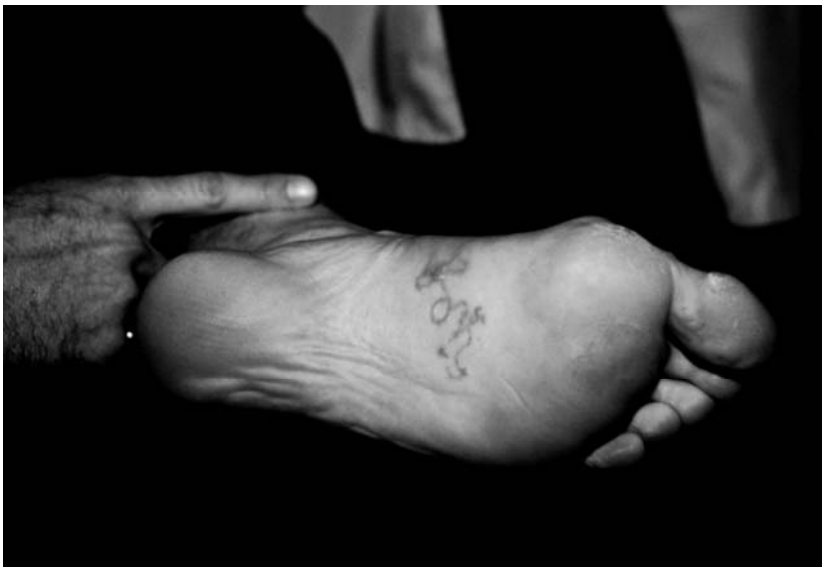
Opening pressure 25 cm of $H_2O$
The laboratory received 2 mls clear colourless fluid
White cells – 800 (99% lymphocytes)
Red cells – 10
No organisms seen
No growth on culture
Protein 3.5 g/l
CSF glucose 2.8 mmol/l
Blood glucose 5.3 mmol/l

a) What is the differential diagnosis of infection? (give 2)

b) Name 2 non-infectious causes of these findings?

c) What further tests (give 2) and treatment would you advise?

7. A 19 year old gap year student has recently been travelling in Mexico. He has been keeping costs down by sleeping on the beach. He presents with an extremely itchy rash that is unresponsive to anti-histamine given orally and topically.

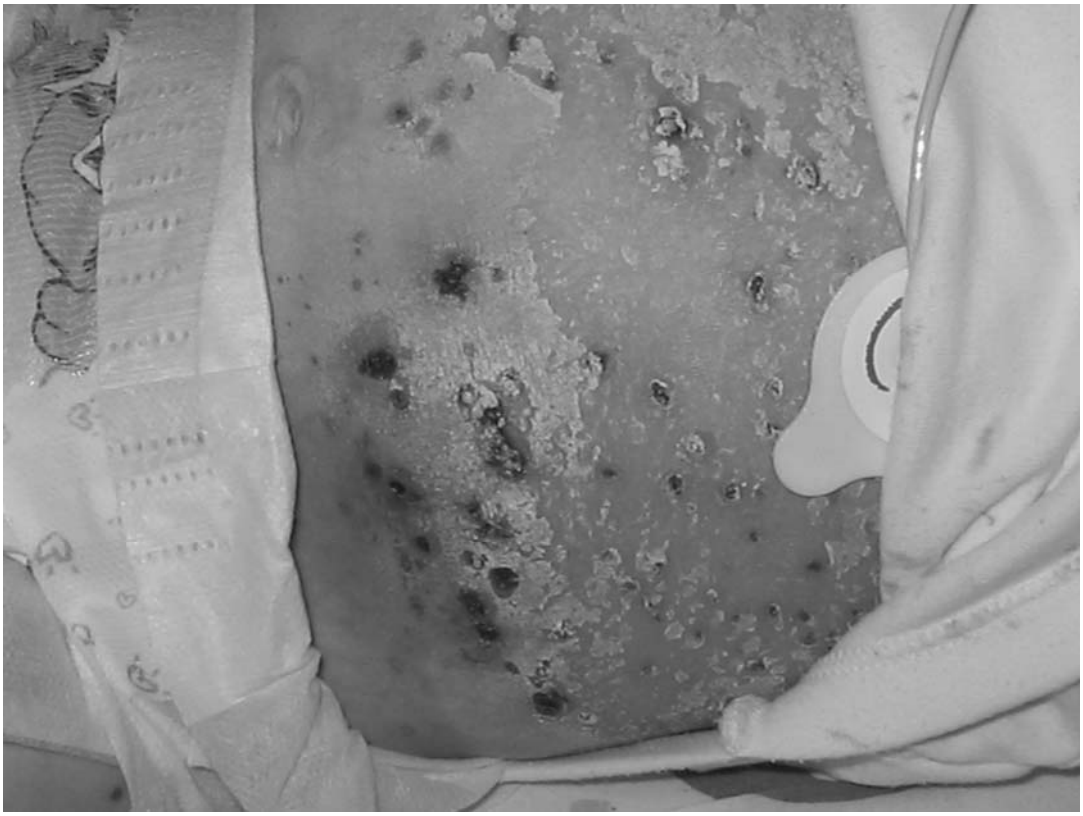


a) What is the diagnosis?

b) List the two species that cause this clinical picture?

c) Give your management strategy?

8. This 9-month-old child has chicken pox, but the GP is concerned about super-infection.

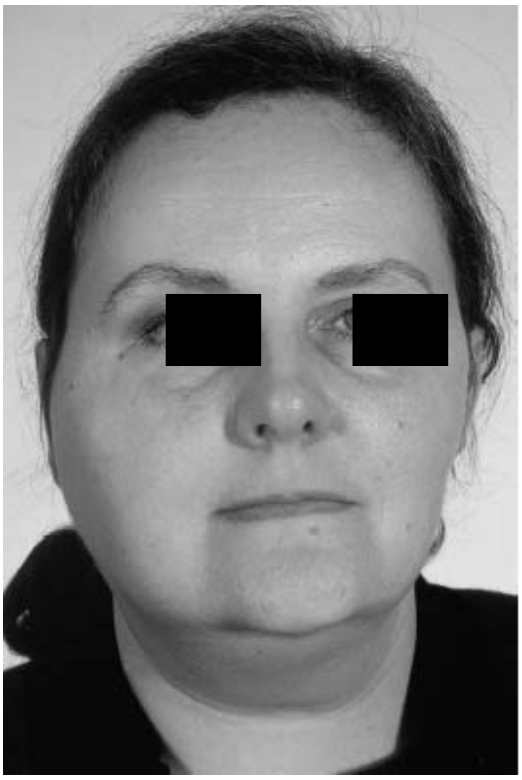


a) Which organisms classically cause super-infection? (give 2)

b) What complications result from these super-infections? (give 2)

c) What empiric treatment would you advise when super-infection is present?

9. A 22 year old college student developed fever, headache and soon thereafter presented to her GP with sign depicted in the photograph below.

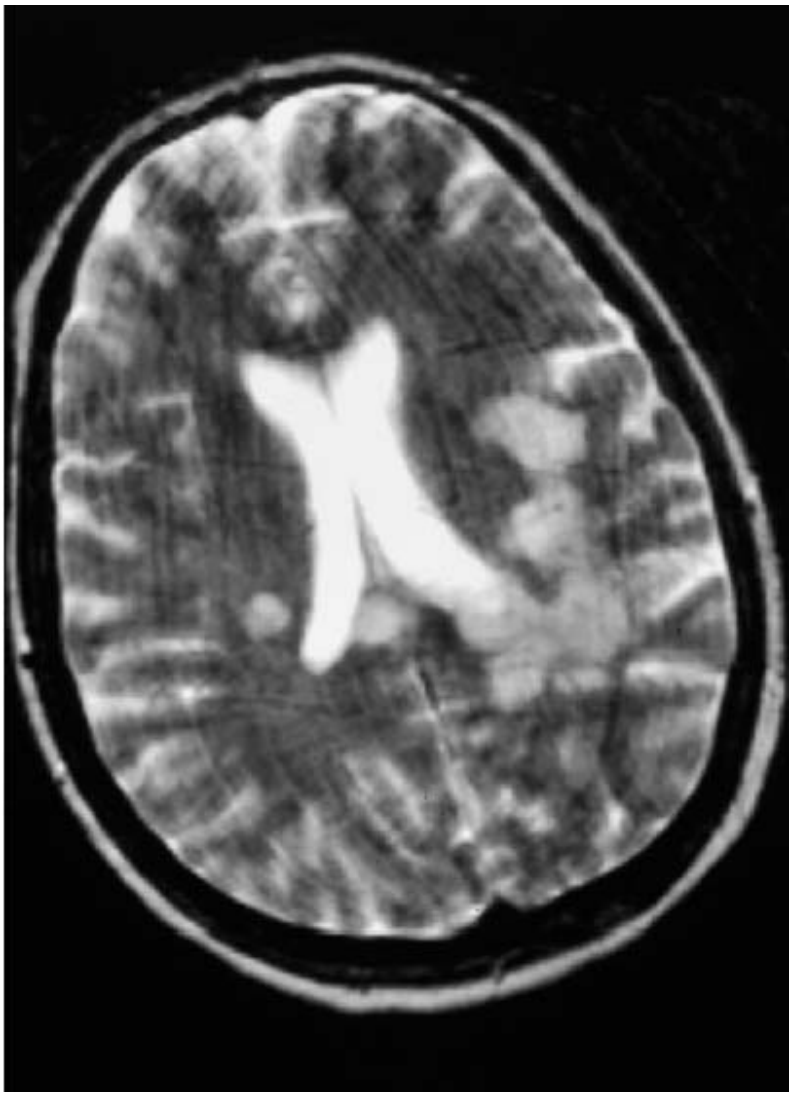


a) What is the likely diagnosis?

b) What test would you advise to confirm this diagnosis?

c) How do you advise this case be managed and what are the implications for her and the college?

10. This 28 year-old woman developed primary HSV genital infection, some 4 weeks prior to the onset of right hemi-paresis and aphasia.



a) What is the likely diagnosis, based upon the findings of the MRI scan shown and the clinical history?

b) What is the likely clinical outcome of the disease?

c) What circumstances are associated with this condition? (give 2)

## Section B: Journal article evaluation questions

*Answer **two** of the following questions. Your answers should be concise. You should justify your answers by reference to the article where ever possible. In addition, you should include your knowledge of relevant literature when this is available.*

### Question 1

**Prospective evaluation of BD Probtec strand displacement amplification system for diagnosis of tuberculosis in non-respiratory and respiratory samples. TD McHugh et al. Journal of Medical Microbiology 2004 53: 1215-1219**

a) Describe the approach used to selecting samples for study. What are the weaknesses/strengths of the approach that the authors have used? How may this have affected the results obtained? Suggest how sampling may have been improved.

b) What are positive and negative predictive values and outline how the PPV and NPV should be interpreted in this study.

c) If you were establishing a molecular diagnostic service for mycobacterial diagnosis, how would these data affect the way in which you introduced it?

### Question 2

**Pépin J *et al. Clostridium difficile*-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. *JAMC* 2004; 171: 466-72.**

Carefully read this study and answer the following questions. You should refer to key statements used by the authors to justify your answers.

a) In the context of this study what is ascertainment bias – was it present here?

b) Comment on the definition of CDAD.

c) Discuss the observation that outcome appeared to worse in metronidazole recipients as compared with those treated with vancomycin.

d) Comment on the data in Table 3 that have not been discussed/explained by the authors.

## Question 3

***Christ-Crain* M *et al.* Effect of procalcitonin (PCT)-guided treatment on antibiotic use and outcome in lower respiratory tract infections: cluster-randomised, single-blinded intervention trial. *Lancet* 2004; 363: 600-7.**

Carefully read this study and answer the following questions. You should refer to key statements used by the authors to justify your answers.

a) Explain what is a cluster-randomised, single-blinded study. Briefly, describe the advantages and disadvantages of this design as applied to this study (detailed statistical knowledge is not required)?

b) Comment on the intention to use antibiotics and on interventions other than PCT testing that could be used to achieve similar effects.

c) Comment on the implications of this study for clinical practice.

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# Prospective evaluation of BDProbeTec strand displacement amplification (SDA) system for diagnosis of tuberculosis in non-respiratory and respiratory samples

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Nucleic acid amplification techniques (NAATs) have been demonstrated to make significant improvements in the diagnosis of tuberculosis (TB), particularly in the time to diagnosis and the diagnosis of smear-negative TB. The BD ProbeTec strand displacement amplification (SDA) system for the diagnosis of pulmonary and non-pulmonary tuberculosis was evaluated. A total of 689 samples were analysed from patients with clinically suspected TB. Compared with culture, the sensitivity and specificity for pulmonary samples were 98 and 89 %, and against final clinical diagnosis 93 and 92 %, respectively. This assay has undergone limited evaluation for non-respiratory samples and so 331 non-respiratory samples were tested, identifying those specimens that were likely to yield a useful result. These were CSF ($n = 104$), fine needle aspirates ($n = 64$) and pus ($n = 41$). Pleural fluid ($n = 47$) was identified as a poor specimen. A concern in using the SDA assay was that low-positive samples were difficult to interpret; 7·8 % of specimens fell into this category. Indeed, 64 % of the discrepant results, when compared to final clinical diagnosis, could be assigned as low-positive samples. Specimen type did not predict likelihood of a sample being in the low-positive zone. Although the manufacturers do not describe the concept of a low-positive zone, we have found that it aids clinical diagnosis.

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## INTRODUCTION

Nucleic acid amplification techniques (NAATs) have been established in the diagnosis of tuberculosis, making significant improvements in the time to diagnosis with benefits both for the individual patient and in the provision of services. It has been shown that the addition of molecular techniques to our mycobacterial diagnostic service reduced the time to confirmed diagnosis from a mean of 37·5 to 22 days (Davies *et al.*, 1999). Rapid diagnosis reduces the costs of management of multidrug-resistant-tuberculosis, with estimated savings of between £50 000 and £150 000 per annum being achieved following early detection (Drobniewski *et al.*, 2000). Many NAATs have been employed successfully for the detection of *Mycobacterium tuberculosis* (Huggett *et al.*, 2003). Several commercial systems exist and are in routine use, for example AMPLICOR (Roche Diagnostics; Beavis *et al.*, 1995), Gen-Probe (Gene-Probe; Gamboa *et al.*, 1998), ligase chain reaction (Abbot; Lindbrathen *et al.*, 1997), RealArt *M. tuberculosis* PCR kit (Artus) and the BDProbeTec SDA system (Becton Dickinson; Down *et al.*, 1996) adopted in this study. The BDProbeTec is a semi-automated real-time system which allows simultaneous amplification and detection of *M. tuberculosis* target DNA IS*6110* using amplification primers and fluorescently labelled probe.

Commercial systems provide benefits of quality assurance for reagents, user-friendly formats and automated handling of large numbers. However, they are often only fully evaluated for specific clinical specimens and indications, and amplification methods for *M. tuberculosis* are commonly only validated on smear-positive respiratory samples. Non-pulmonary tuberculosis represents a tougher diagnostic challenge. We therefore performed a prospective evaluation of the BDProbeTec system for diagnosis of both non-pulmonary and pulmonary samples. The BDProbeTec system is marketed as a qualitative system, providing a positive or negative result. However, numerical data are produced

Abbreviations: FNA, fine needle aspirates; NAAT, nucleic acid amplification techniques; SDA, strand displacement amplification; TB, tuberculosis.

and a positive result has a value of >3400 MOTA (Metric Other Than Acceleration). During the course of this evaluation we noted that the samples with positive SDA results <40 000 MOTA were responsible for the majority of diagnostic anomalies and so we extended the analysis to investigate the results in this 'low-positive zone'.

## METHODS

**Specimens.** Specimens were obtained prospectively from those submitted routinely to the Molecular Diagnostic Service of the Royal Free Hospital between and including January 2001 and December 2002. Molecular analysis was performed on all those patients in whom the clinician managing the case suspected tuberculosis and requested a *M. tuberculosis* NAAT.

**Microbiological methods.** Specimens were stored at 4 °C prior to decontamination. Respiratory specimens were treated with an equal volume of *N*-acetyl-L-cysteine (NALC)-NaOH (final concentration 2 %) for 15 min at room temperature and were neutralized with sterile phosphate buffer (0·067 M, pH 6·8). After centrifugation at 3000 ***g*** for 30 min, the pellet was resuspended in 10 ml sterile distilled water and further centrifuged at 3000 ***g*** for 30 min. Half the deposit was inoculated into the culture medium and the remaining half subjected to SDA investigation.

Liquid non-respiratory specimens were centrifuged at 3000 ***g*** for 30 min. Half the resultant pellet was inoculated into culture medium and half submitted to SDA. Similarly, following homogenization in a sterile Griffiths tube homogenizer, tissues were divided into two equal portions. Pellets were resuspended in 2·0 ml phosphate buffer and subjected to both culture and SDA analysis.

All specimens were screened microscopically after concentration using the Auramine stain with positive results confirmed using Ziehl–Neelsen staining. Culture was performed using the MBAlert 3D (bioMérieux) liquid culture system following the manufacturer's instructions.

**Molecular methods.** Specimens were treated according to the manufacturer's instructions. In brief, a volume of 100–500 µl decontaminated specimen was washed with 1 ml wash buffer (BD-SDA buffer 1) prior to centrifugation in a microfuge at 12 200 ***g*** for 3 min. The supernatant was decanted and mycobacteria killed by heating the pellet to 105 °C for 30 min. DNA was released from cells in the deposit by resuspension in 100 µl lysis buffer (BD-SDA buffer 2), followed by sonication in a soft polymer tube at 65 °C for 45 min. Samples were then neutralized by addition of 600 µl BD-SDA neutralization buffer.

A 150 µl aliquot of the DNA extract was added to a priming well containing dehydrated primers and probes, in the microtitre plate supplied. To ensure complete rehydration of reagents the plate was incubated at room temperature for 20 min. This priming mix was then incubated at 72·5 °C for 10 min. In a separate microtitre plate, amplification wells containing enzymes, dNTPs and buffer were activated by heating to 54 °C for 10 min. Amplification was activated by the addition of 100 µl of the priming mix to the corresponding amplification well and mixing. Plates were then transferred to the ProbeTec analyser.

Each assay run includes positive and negative controls and each test well contains an internal control. Samples that gave a fluorescence reading of >3400 MOTA were regarded as positive.

**Clinical data.** Specimens were only entered into the study if a minimal clinical dataset was available (patient identifiers and specimen date, sample type and anatomical site, SDA assay, smear and culture results, symptomatic and radiological evidence of tuberculosis). A final clinical diagnosis of tuberculosis was confirmed by cross-reference to the Statutory Infectious Diseases Notification records.

**Statistical analysis.** Analysis was performed on the basis of each specimen or sample and not on the basis of patient. Sensitivity and specificity of the assay was calculated for each specimen type using the formula described previously by Motulsky (1995).

## RESULTS

Over the course of this study a total of 358 respiratory and 331 non-respiratory specimens from 307 patients were investigated. Multiple specimens (2–5/patient) were received from 132 patients, these included sampling of the same site on separate occasions as well as sampling of multiple sites on the same occasion. Each specimen was treated separately in this analysis, as a specimen represents an independent diagnostic event. In assigning a final diagnosis all specimens were considered together with microbiological and clinical data. The respiratory specimens consisted of sputum (169) and bronchial washings/lavages (189). Non-respiratory specimens included cerebrospinal fluid (CSF; 104), fine needle aspirates (FNA; 64), ascitic fluid (18), pus (41), pleural fluid (47), fluids (17) and other specimens (40).

The results of culture, SDA, acid-fast bacilli (AFB) smear and final clinical diagnosis are summarized in Tables 1 and 2. Ninety-five patients had a clinical diagnosis of pulmonary tuberculosis and 69 had a diagnosis of extra-pulmonary tuberculosis. On culture, 83/358 respiratory samples were positive for *M. tuberculosis* (Table 1) a further 14 samples grew non-tuberculosis mycobacteria (NTM), these were

**Table 1.** SDA, culture, smear and clinical diagnosis (CD) of 358 respiratory samples (189 bronchial washings, 169 sputum)

| Mtb SDA result | Culture-positive | | | | Culture-negative | | | |
|---|---|---|---|---|---|---|---|---|
| | Smear-positive | | Smear-negative | | Smear-positive | | Smear-negative | |
| | CD: not TB | CD: TB | CD: not TB | CD: TB | CD: not TB | CD: TB | CD: not TB | CD: TB |
| Positive | 0 | 48 | 0 | 33 | 0 | 2 | 19 | 9 |
| Negative | 0 | 0 | 0 | 2 | 0 | 0 | 240 | 5 |

**Table 2.** SDA, culture, smear and clinical diagnosis (CD) of 331 non-respiratory samples

| Specimen type | Mtb SDA result | Culture-positive | | | | Culture-negative | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Smear-positive | | Smear-negative | | Smear-positive | | Smear-negative | |
| | | CD: not TB | CD: TB | CD: not TB | CD: TB | CD: not TB | CD: TB | CD: not TB | CD: TB |
| Ascitic fluid ($n = 18$) | Positive | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| | Negative | 0 | 0 | 0 | 2 | 0 | 0 | 12 | 2 |
| CSF ($n = 104$) | Positive | 0 | 1 | 0 | 1 | 0 | 0 | 3 | 2 |
| | Negative | 0 | 0 | 0 | 0 | 0 | 0 | 91 | 6 |
| Fluids ($n = 17$) | Positive | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 |
| | Negative | 0 | 0 | 0 | 2 | 0 | 0 | 10 | 1 |
| FNA ($n = 64$) | Positive | 0 | 3 | 0 | 5 | 0 | 1 | 4 | 0 |
| | Negative | 0 | 0 | 0 | 3 | 0 | 1 | 46 | 1 |
| Pleural fluid ($n = 47$) | Positive | 0 | 0 | 1 | 2 | 0 | 0 | 3 | 0 |
| | Negative | 0 | 0 | 3 | 4 | 0 | 0 | 29 | 5 |
| Pus ($n = 41$) | Positive | 1 | 1 | 0 | 10 | 1 | 1 | 2 | 2 |
| | Negative | 2 | 1 | 1 | 1 | 1 | 0 | 16 | 1 |
| Others ($n = 40$) | Positive | 0 | 1 | 0 | 1 | 0 | 0 | 4 | 4 |
| | Negative | 0 | 0 | 1 | 1 | 1 | 0 | 24 | 3 |

*Mycobacterium avium* (1), *Mycobacterium kansasii* (3), *Mycobacterium fortuitum* (5), *Mycobacterium xenopi* (3) and two unidentified environmental mycobacteria. Of the non-respiratory samples 54/331 were culture-positive for *M. tuberculosis* (Table 2) and one specimen grew *M. avium*. For respiratory samples 111/358 (31 %) were positive by SDA and for non-respiratory samples 60/331 (18 %) were positive by SDA.

Using the manufacturer's cut-off of 3400 MOTA, the sensitivity and specificity for respiratory samples was 98 and 89 %, respectively, compared to culture (Table 3). When compared to clinical diagnosis the sensitivity was reduced but the specificity increased (Table 3).

An analysis of assay performance was undertaken for each non-respiratory specimen type where there were adequate sample numbers for valid interpretation (Table 3). When compared to culture, CSF samples gave good sensitivity and specificity (100 and 95 %). The assay characteristics for FNA and pus specimens were adequate to provide useful data, however, pleural fluid samples had very poor sensitivity (30 %).

All other non-respiratory sites were evaluated on the basis of potential clinical relevance and individual case histories.

Review of the false-positive results identified 42 of which 27 (64 %) fell between <3400 MOTA and <40 000 MOTA, designated the low-positive zone. The remaining 15 had values above 40 000 MOTA. Forty-six per cent of the low-positive zone specimens (27/58) were discrepant and of these 15 were respiratory specimens. The remaining 12 non-respiratory specimens came from a range of sites with no single type predominating (pleural fluid, 3; fluid, 2; FNA, 2; ascitic fluid, 1; CSF, 3; pus, 1).

**Table 3.** Sensitivity (sens.), specificity (spec.), positive predictive value (PPV) and negative predictive value (NPV) of Mtb SDA for various specimen types compared to smear, culture and final diagnosis for each sample

| Sample | Smear | | | | Culture | | | | Final diagnosis | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sens. (%) | Spec. (%) | PPV | NPV | Sens. (%) | Spec. (%) | PPV | NPV | Sens. (%) | Spec. (%) | PPV | NPV |
| Respiratory specimens | 100 | 80 | 45 | 100 | 98 | 89 | 73 | 99 | 93 | 92 | 81 | 97 |
| CSF* | 100 | 94 | 14 | 100 | 100 | 95 | 29 | 100 | 40 | 97 | 57 | 94 |
| FNA | 80 | 85 | 31 | 98 | 73 | 91 | 62 | 94 | 64 | 92 | 69 | 90 |
| Pleural fluid | 100 | 87 | 0 | 100 | 30 | 92 | 50 | 83 | 18 | 89 | 33 | 78 |
| Pus | 100 | 58 | 22 | 83 | 71 | 75 | 66 | 78 | 78 | 83 | 78 | 87 |

*Note of caution: of 104 CSF samples tested only two were positive.

## DISCUSSION

The purpose of NAAT in the diagnosis of tuberculosis is to identify patients requiring treatment rapidly. Using the methods in this way means that applying them to specimens that are smear-positive provides the best diagnostic yield. However, such a policy is less rational than it first appears, as often the clinical presentation, examination and radiology mean that a positive smear is adequate to make a presumptive diagnosis. This is especially true when infections with non-tuberculosis mycobacteria such as *M. avium intracellulare* are less likely with patients on highly active antiretroviral therapy (HAART). We have previously shown that results from smear-negative patients have the biggest impact on treatment decisions (Conaty *et al.*, 2004). Thus, the application of NAAT to specimens that are smear-negative or from a non-pulmonary source is important.

The BDProbeTec SDA system is now well established in the diagnosis of respiratory tuberculosis and the data presented here reflect both the manufacturer's data and that of other groups (Barrett *et al.* 2002; Mazzarelli *et al.*, 2003). For respiratory samples, SDA improved sensitivity (Table 3) over smear alone, which has a sensitivity of 58 % against culture and 53 % against final clinical diagnosis. In this study, the specificity of the SDA was notably low (89 %), when compared to other NAATs, although it is comparable to the test characteristics published by the manufacturers (91 %). We have previously reported that there is cross-reactivity between the target (IS*6110*) and mycobacteria other than tuberculosis (McHugh *et al.*, 1997), such cross-reactivity may contribute to the decreased specificity of the test. This view is supported by the observation that specificity against culture is good in those samples that are likely to have fewer competing bacteria (CSF, FNA and pleural fluid) as compared to pus and respiratory specimens. Of course it should be noted that 'final diagnosis' includes an element of bias, as the SDA result often contributes to the final diagnosis.

In this study we have identified samples that are low-positive ($>$3400–$<$ 40 000 MOTA), which formed 7·8 % of all specimens. Of the discrepant results, when compared to final clinical diagnosis, 64 % could be assigned to the low-positive zone. There were 54 samples in this low-positive zone, of which 46 % were discrepant. Specimen type did not predict the likelihood of a sample being in the low-positive zone, 15/27 discrepant results were respiratory samples, the remaining 12 samples were from a wide range of non-respiratory sites with no single site being over-represented ($P > 0{\cdot}05$). Although the manufacturers do not describe the concept of a low-positive zone, we have found that its introduction has been an aid to interpretation for clinical diagnosis. These samples represent a small proportion of the total, but our experience is that they demand a disproportionate effort to achieve a satisfactory final diagnosis. The implications for a patient of being treated inappropriately for tuberculosis are substantial. Thus, we propose that any sample with results in the low-positive zone is automatically repeated and a further specimen sought. Only if all three results are concordant should a result be reported. The biological significance of the false-positive results in the low-positive zone remains unclear, the presence of low levels of *M. tuberculosis* DNA as a result of environmental exposure or non-viable infection are both possibilities. The critical point is that these patients did not go on to develop clinical disease which emphasizes the need to view the molecular test result within the context of the whole clinical picture.

In this 2-year review of our practice we have identified those non-respiratory samples for which an SDA result may be interpreted with some confidence. CSF is a critical specimen with good sensitivity and specificity as compared to smear and culture (Table 3). It should be noted that this analysis is based on 2/104 positive samples. The manufacturers report a sensitivity of 125 c.f.u. $ml^{-1}$ for the SDA assay, this is tenfold greater than the likely number of organisms in CSF, thus the test is being used at the limit of its analytical sensitivity in this context, and this may contribute to the poor sensitivity in comparison to final diagnosis (40 %). This reflects the importance of non-microbiological markers, such as CSF pleocytosis and biochemistry in the diagnosis of TB meningitis and identifies a role for the SDA in rapid diagnosis. Recently, Johansen *et al.* (2004) described a modified extraction protocol and subsequent re-evaluation of the 3400 MOTA cut-off for CSF samples; this is a promising development that may increase the sensitivity for this important specimen. The low sensitivity but high specificity of the SDA for FNA samples reflects low organism numbers often found in these specimens, however, the good specificity makes the test very useful clinically. Conversely, applying the SDA to pus samples showed good sensitivity but poor specificity, thus, although a negative result may be helpful, the poor specificity suggests that SDA should be applied to pus samples with caution. Pleural fluid is known to be a poor sample for smear and culture testing due to sampling and dilution effects (Valdes *et al.*, 2003). This is also the case for SDA testing, sensitivity of this test for pleural fluids is so low that the test is of little value.

This is the largest prospective review of NAAT for the diagnosis of non-respiratory samples. As a result we are able to focus our resources on those specimens that are most likely to yield clinically relevant results. We confirm the value of NAAT techniques in diagnosis from respiratory samples, and particularly enhanced sensitivity from smear-negative specimens (Conaty *et al.*, 2004). Also, we have demonstrated that certain non-respiratory samples are appropriate for NAAT diagnosis but there is a significant risk of biological false-positive results and so these tests must be interpreted with caution.

## ACKNOWLEDGEMENTS

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# *Clostridium difficile*-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity

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**Abstract**

**Background:** Recent reports suggest that *Clostridium difficile* colitis may be evolving into a more severe disease. During the second half of 2002 we noted an increase in the number of patients with severe *C. difficile*-associated diarrhea (CDAD) in our institution. We describe cases of CDAD at our institution over a 13-year period and investigate changes in illness severity.

**Methods:** We undertook a retrospective chart review of all cases of CDAD diagnosed at the Centre hospitalier universitaire de Sherbrooke from Jan. 1, 1991, to Dec. 31, 2003. Because the hospital serves a well-defined population of Quebec, we were also able to calculate population-based incidence during this period. We abstracted data on individual patients from patient charts and from hospital and pharmacy computer databases. We defined cases of CDAD as having a positive *C. difficile* cytotoxicity assay result, or endoscopic or histopathological evidence of pseudomembranous colitis. A case was considered complicated if one or more of the following was observed: megacolon, perforation, colectomy, shock requiring vasopressor therapy, or death within 30 days after diagnosis.

**Results:** A total of 1721 cases of CDAD were diagnosed during the study period. The incidence increased from 35.6 per 100 000 population in 1991 to 156.3 per 100 000 in 2003; among patients aged 65 years or more, it increased from 102.0 to 866.5 per 100 000. The proportion of cases that were complicated increased from 7.1% (12/169) in 1991–1992 to 18.2% (71/390) in 2003 ($p < 0.001$), and the proportion of patients who died within 30 days after diagnosis increased from 4.7% (8/169) in 1991–1992 to 13.8% (54/390) in 2003 ($p < 0.001$). A high leukocyte count ($20.0 \times 10^9$/L or greater) and an elevated creatinine level (200 μmol/L or greater) were strongly associated with adverse outcomes: in 2003, 45 (40.9%) of 110 patients with a high leukocyte count or creatinine level, or both, had complicated CDAD and 28 (25.5%) died within 30 days after diagnosis. After adjustment for age and other confounding factors, patients initially given oral vancomycin therapy had a risk of progression to complicated CDAD that was 79% lower than the risk among patients initially treated with metronidazole (adjusted odds ratio 0.2, 95% confidence interval 0.06–0.8, $p = 0.02$).

**Interpretation:** An epidemic of CDAD with an increased case-fatality rate has had important consequences on the elderly population of our region. Our observational data suggest that the equivalence of vancomycin and metronidazole in the treatment of CDAD needs to be questioned.

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*Clostridium difficile* is an important cause of diarrhea in industrialized countries and the leading cause of infectious diarrhea among patients in hospital.[1–6] In the second half of 2002 we noted an increase in the number of patients with fulminant *C. difficile* colitis in our institution who required an emergency colectomy. Informal discussions with clinicians from other hospitals in southern Quebec suggested that this phenomenon was occurring in other cities as well. To investigate whether this was related merely to an increase in the number of cases of *C. difficile*-associated diarrhea (CDAD) or to an increase in the proportion of cases that developed severe colitis, we undertook a retrospective study of all cases of CDAD diagnosed at our institution over a 13-year period and investigated changes in illness severity.

## Methods

We undertook a retrospective chart review of all cases of CDAD diagnosed at the Centre hospitalier universitaire de Sherbrooke (CHUS) from Jan. 1, 1991, to Dec. 31, 2003. This 683-bed hospital provides secondary and tertiary care to the inhabitants of the Estrie region (Eastern Townships) of the province of Quebec. Because the hospital serves a well-defined population of Quebec, we were also able to calculate population-based incidence during this period. The Estrie population increased over the study period, from 274 670 in 1991 to 294 058 in 2003, according to annual estimates made by the regional health authority from the most recent census data. About half of the population lives in Sherbrooke (134 981 in 1991 and 146 559 in 2003). The CHUS clinical microbiology laboratory is the sole regional laboratory performing *C. difficile* toxin assays for all but 2 primary

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care facilities, which send their specimens to laboratories outside the region. The CHUS laboratory receives nearly all requests for *C. difficile* toxin assays for residents of Sherbrooke, and about 60% of those for residents in the Estrie region who live outside of Sherbrooke.

The same *C. difficile* toxin B cytotoxin assay was used throughout the study period, using either Vero cells or MRC-5 cells, with readings after 24 and 48 hours of incubation and neutralization with *C. difficile* antitoxin (Bartels Inc., Issaquah, Wash.). Until mid-1996, the cytotoxin assay was routinely performed on all stool samples received for culture; afterward, it had to be specifically requested. Since the end of 1990, patient records at the CHUS, including those of patients at other institutions whose stool specimens were analyzed at the CHUS laboratory, have been entered into a computer database, with the exception of medical and nursing notes. We chose Jan. 1, 1991, as the start of the observation period because we could from then on identify nearly all cases (both inpatients and outpatients) with a positive cytotoxin assay result through the hospital computerized medical records. In addition, we searched the hospital discharge database for all patients who died or left the hospital with a recorded diagnosis of pseudomembranous colitis, antibiotic-associated colitis or *C. difficile* colitis.

We defined cases of CDAD as those meeting at least one of the following criteria: a positive cytotoxin assay result; endoscopic evidence of pseudomembranous colitis; and histopathological evidence of pseudomembranous colitis on a specimen obtained during endoscopy, colectomy or autopsy. We excluded patients with a discharge diagnosis of pseudomembranous colitis, *C. difficile* colitis or antibiotic-associated colitis but without any supporting evidence. Two episodes of CDAD occurring in the same patient were considered distinct events if they occurred more than 3 months apart; an episode occurring within 3 months of a prior one was considered a relapse. We defined CDAD as hospital-acquired if the patient was in hospital when the inciting antibiotics were administered. A case of CDAD was considered to be complicated if the patient died within 30 days after the diagnosis of CDAD or if any of the following occurred: megacolon, perforation, colectomy or shock requiring vasopressor therapy.

We reviewed patient records from the CHUS database. For patients whose stool specimens were sent to the CHUS without being seen by one of its staff physicians, only the information that accompanied the specimen (patient name, address, sex, date of birth) was available; this was used to calculate incidence rates, but such patients did not contribute to measures of disease severity. For patients seen at the CHUS (both inpatients and outpatients), we abstracted clinical and laboratory data, including the use of antibiotics, tube feeding or surgery during the 2 months before the diagnosis of CDAD, evidence of immunosuppression (defined as the presence of leukemia, lymphoma, HIV infection, neutropenia, organ transplantation or use of immunosuppressive drugs), antibiotics used for the treatment of CDAD and evidence of complications. Peak leukocyte count and creatinine level corresponded to the highest value within 1 week after diagnosis of CDAD.

We also tracked the use of antibiotics among all CHUS inpatients, regardless of whether they had CDAD, by reviewing data from a pharmacy database for 1999–2003. For each antibiotic, we estimated the number of patient-days of use by dividing the total number of grams dispensed during the year by the defined daily dosage (the latter determined by consensus of local infectious diseases consultants).

For statistical analysis, proportions were compared with the Yates-corrected $\chi^2$ test or Fisher's exact test when numbers were small. Unconditional logistic regression was used for multivariate analyses. Models were built up sequentially, starting with the variable most strongly associated with the outcome and continuing until no other variable reached significance or altered the odds ratios of variables already in the model. When the final model was reached, each variable was dropped in turn to assess its effect. Different models were compared using the likelihood ratio test.

## Results

A total of 1721 new cases of CDAD were identified during the 13-year study period, 1658 (96.3%) of which had a positive cytotoxin assay result. Of those without a positive cytotoxin assay result, 59 had endoscopic changes typical of pseudomembranous colitis, and 4 were diagnosed on histopathological grounds. Fig. 1 shows the incidence of CDAD among residents of Sherbrooke during the study period. The annual incidence increased from 35.6 per 100 000 population in 1991 to 156.3 per 100 000 in 2003. Among residents in the Estrie region outside of Sherbrooke, the incidence remained stable from 1991 to 2002 (22.2 and 25.2 per 100 000 respectively) and increased in 2003 (92.2 per 100 000); however, incidence rates are probably underestimated, since referral of cases and specimens was incomplete and may have changed over time. Age-specific incidence rates in Sherbrooke are also shown in Fig. 1. The incidence among children decreased after 1996, when the cytotoxin assay had to be specifically requested. Among people aged 18–64 years, the incidence increased only in 2003. Among people aged 65 years or more, the annual incidence increased 10-fold during the study period, up to 866.5 cases per 100 000 in 2003; the incidence was 1681 per 100 000 among those aged 80 years or more.

The case-fatality rate and the proportion of cases that were considered complicated more than doubled over time, as measured among the 1675 new cases for whom enough information was available to assess these outcomes (Table 1). The absolute number of patients who had megacolon, perforation or shock requiring vasopressor therapy, who needed a colectomy or who died within 30 days after diagnosis of CDAD increased dramatically, from 6–10 such cases per year during 1991–1998 to 71 cases in 2003. After adjustment for age, sex, initial treatment of CDAD, immune status, and tube feeding and surgery in the 2 months preceding diagnosis, cases diagnosed in 2003 had a higher likelihood of having complicated CDAD than those diagnosed in previous years. In these models, the peak leukocyte counts and creatinine levels were not included because these parameters are clearly on the causal pathway between year of diagnosis and the outcomes.

From 1991 to 2002 the proportion of cases in which complicated CDAD developed and the proportion of cases in which death occurred within 30 days after diagnosis were 25.4% (68/268) and 19.0% (51/268) respectively among patients with a high leukocyte count ($20.0 \times 10^9$/L or greater), an elevated creatinine level (200 μmol/L or greater) or both.

The corresponding proportions among patients with lower leukocyte counts and creatinine levels were 6.0% (40/666) and 4.2% (28/666). In 2003, the proportion of cases with complicated CDAD and the proportion of cases in which death occurred within 30 days after diagnosis increased, both among patients with high leukocyte counts, creatinine levels or both (40.9% [45/110] and 25.5% [28/110] respectively) and among those with lower leukocyte counts and creatinine levels (12.3% [25/204] and 11.3% [23/204] respectively). In 2003, of the 110 patients with high leukocyte counts, creatinine levels, or both, 87 were initially given metronidazole; complicated CDAD developed in 34 (39.1%) of them, including 20 (23.0%) who died within 30 days after diagnosis. Only 2 such patients were initially given vancomycin, none of whom had complicated CDAD.

Factors associated with complicated CDAD, in uni-

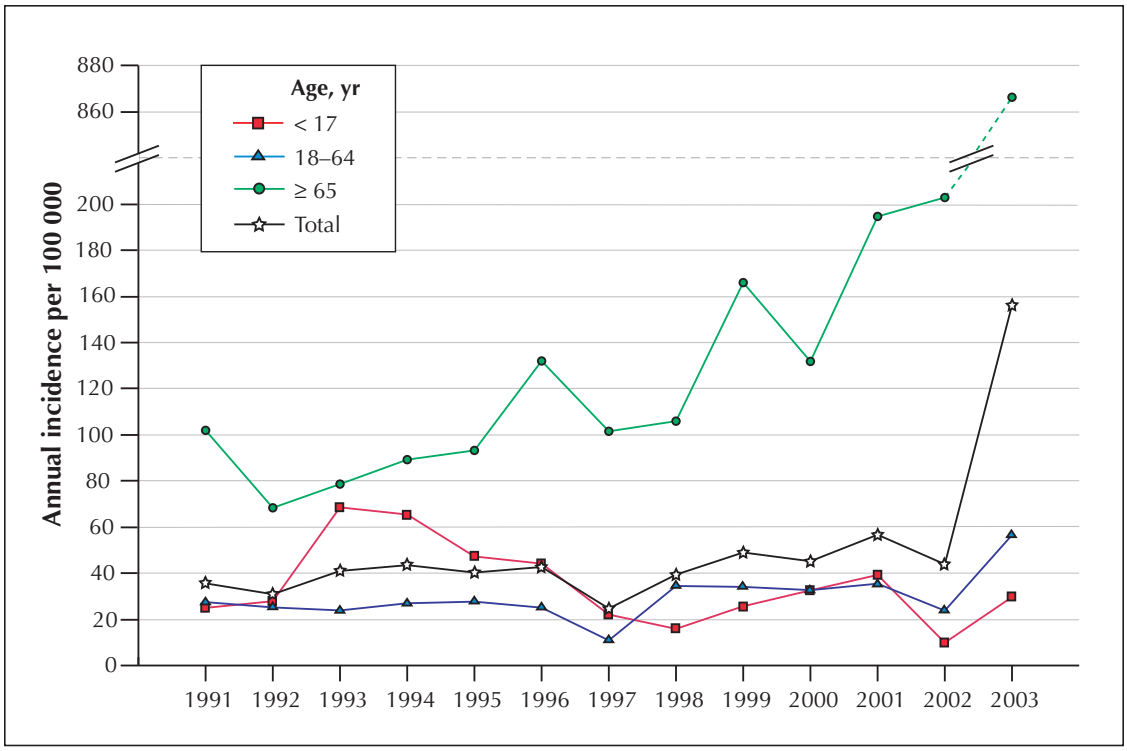


**Fig. 1: Annual incidence (per 100 000 population) of *Clostridium difficile*-associated diarrhea (CDAD) in Sherbrooke, Que., 1991–2003.**

**Table 1: Patients with *Clostridium difficile*-associated diarrhea (CDAD) in the Estrie region of Quebec who died within 30 days after diagnosis or who had complicated CDAD, 1991–2003**

| Period | No. of patients with CDAD* | No. (%) who died within 30 days after diagnosis | Adjusted OR (95% CI)† | No. (%) who had complicated CDAD‡ | Adjusted OR (95% CI)† |
|---|---|---|---|---|---|
| 1991–1992 | 169 | 8 (4.7) | 1.0 | 12 (7.1) | 1.0 |
| 1993–1994 | 217 | 11 (5.1) | 1.7 (0.5–5.3) | 14 (6.5) | 1.0 (0.4–2.7) |
| 1995–1996 | 215 | 13 (6.0) | 1.6 (0.5–5.0) | 17 (7.9) | 0.9 (0.3–2.2) |
| 1997–1998 | 192 | 11 (5.7) | 1.1 (0.4–3.7) | 13 (6.8) | 0.6 (0.3–1.7) |
| 1999–2000 | 248 | 19 (7.7) | 1.5 (0.5–4.6) | 28 (11.3) | 1.2 (0.5–2.9) |
| 2001–2002 | 244 | 21 (8.6) | 1.6 (0.5–4.7) | 28 (11.5) | 1.1 (0.5–2.5) |
| 2003 | 390 | 54 (13.8) | 3.0 (1.1–8.4) | 71 (18.2) | 2.2 (1.0–4.9) |
| *p* value | | < 0.001§ | < 0.001¶ | < 0.001§ | 0.001¶ |

Note: OR = odds ratio, CI = confidence interval.
*Includes only patients for whom enough information was available to assess these outcomes.
†Adjusted for age, sex, initial treatment, immune status, and tube feeding and surgery in the 2 months preceding diagnosis; 1991–1992 was used as the baseline period.
‡Presence of one or more of the following: megacolon, perforation, colectomy, shock requiring vasopressor therapy, death within 30 days after diagnosis.
§$\chi^2$ test for trend.
¶$\chi^2$ test, comparing 2003 with all other years.

variate and multivariate analyses, are shown in Table 2. The population for these analyses included inpatients of the CHUS and the outpatients for whom enough information was available to assess whether they had complicated CDAD. Sex and fever were no longer significant in the multivariate analysis. The independent factors associated with complicated CDAD were age of 65 years or more, hospital-acquired CDAD, tube feeding in the 2 months preceding diagnosis, not having had surgery in the 2 months preceding diagnosis, immunosuppression, a peak leukocyte count of $20.0 \times 10^9$/L or greater and renal failure. After adjusting for these confounding factors, initial treatment with vancomycin was associated with a 79% lower risk of complicated CDAD compared with initial treatment with metronidazole (adjusted odds ratio 0.2, 95% confidence interval 0.06–0.8, $p$ = 0.02). In contrast, initial treatment with metronidazole was not associated with a more favourable outcome compared with no treatment. In these models, which attempted to document short-term correlates of adverse outcomes, peak leukocyte count and creatinine level were included, and year of diagnosis was no longer found to enhance significantly the fit of the models, because part of the enhanced severity of CDAD in recent years was captured by leukocyte count and creatinine level.

Fig. 2 shows the variations over time in the distribution of antibiotics that seemed to provoke CDAD among 1364 patients (515 with community-acquired CDAD and 849 with hospital-acquired infection) for whom information was available on the use of antibiotics in the 2 months preceding diagnosis. Overall, more than two-thirds of the CDAD cases had received cephalosporins in the 2 months before diagnosis. The proportion of patients who had received quinolones increased progressively, up to 55% in 2003, whereas the proportion who had received aminoglycosides decreased. This might to some extent reflect changes in patterns of use of antimicrobial agents and in the distribution of hospital- versus community-acquired cases. Thus, in Table 3 we show the rate of hospital-acquired CDAD per 1000 patient-days of antibiotic use among all CHUS inpatients. For the calculation of this rate, the numerator corresponds to the number of CHUS patients (regardless of city of residence) with hospital-acquired CDAD who received a given class of antibiotic in the 2 months before diagnosis, and the denominator corresponds to the number of patient-days that this class of antibiotic was used among all CHUS inpatients during these years. Compared with the rates in 1999–2002, the rate in 2003 was higher for all classes of antibiotics except cotrimoxazole. Antibiotics associated with the highest risk of CDAD were macrolides, third- and second-generation cephalosporins, clindamycin and quinolones.

**Table 2: Factors associated with complicated CDAD***

| Characteristic | % (no.) of patients with complicated CDAD† | Unadjusted OR (95 %CI) | Adjusted OR (95%CI)‡ |
|---|---|---|---|
| **Sex** | | | |
| Female | 8.7 (82/940) | 1.0 | – |
| Male | 13.7 (101/735) | 1.7 (1.2–2.3) | |
| **Age, yr** | | | |
| ≤ 17 | 1.3 (4/301) | 1.0 | 1.0 |
| 18–64 | 6.0 (38/635) | 4.7 (1.7–13.4)¶ | 1.6 (0.5–5.1) |
| ≥ 65 | 19.1 (141/739) | 17.5 (6.4–47.8)** | 3.4 (1.1–10.3)¶ |
| **Place CDAD acquired** | | | |
| Community | 2.0 (15/765) | 1.0 | 1.0 |
| Hospital | 18.7 (168/900) | 11.5 (6.7–19.7)** | 4.6 (2.4–8.6)** |
| **Surgery in 2 mo preceding diagnosis** | | | |
| No | 10.8 (123/1141) | 1.0 | 1.0 |
| Yes | 13.4 (60/448) | 1.3 (0.9–1.8) | 0.6 (0.4–0.9)¶ |
| **Tube feeding in 2 mo preceding diagnosis** | | | |
| No | 9.5 (135/1416) | 1.0 | 1.0 |
| Yes | 28.5 (43/151) | 3.8 (2.5–5.6)** | 2.4 (1.5–3.9)** |
| **Immunosuppression** | | | |
| No | 9.4 (128/1358) | 1.0 | 1.0 |
| Yes | 21.7 (54/249) | 2.7 (1.9–3.8)** | 2.3 (1.5–3.6)** |
| **Fever (temperature > 38.0°C)** | | | |
| No | 8.8 (52/592) | 1.0 | – |
| Yes | 17.1 (123/718) | 2.1 (1.5–3.0)** | |
| **Peak leukocyte count, × $10^9$/L** | | | |
| < 10.0 | 5.4 (23/423) | 1.0 | 1.0 |
| 10.0–19.9 | 9.8 (62/633) | 1.9 (1.2–3.1)¶ | 1.3 (0.8–2.3) |
| ≥ 20.0 | 33.4 (96/287) | 8.7 (5.4–14.2)** | 4.8 (2.8–8.4)** |
| **Peak creatinine level, µmol/L** | | | |
| < 100 | 6.8 (54/797) | 1.0 | 1.0 |
| 100–199 | 24.1 (78/324) | 4.4 (3.0–6.4)** | 2.2 (1.4–3.5)** |
| ≥ 200 | 32.2 (46/143) | 6.5 (4.2–10.2)** | 3.1 (1.8–5.2)** |
| **Initial antibiotic treatment§** | | | |
| None | 10.4 (41/394) | 1.0 | 1.0 |
| Metronidazole | 13.1 (125/951) | 1.3 (0.9–1.9) | 1.0 (0.6–1.6) |
| Vancomycin | 5.9 (5/85) | 0.5 (0.2–1.4) | 0.2 (0.05–0.8)¶ |
| Metronidazole and vancomycin | 60.0 (9/15) | 12.9 (4.4–38.1)** | 3.7 (0.9–14.1) |

*Presence of one or more of the following: megacolon, perforation, colectomy, shock requiring vasopressor therapy, death within 30 days after diagnosis.
†Numbers vary owing to missing values for some variables.
‡The independent correlates of complicated CDAD are shown in this column along with their adjusted odds ratios. Sex and presence of fever were no longer significant in multivariate analysis and were dropped from this final model.
§Antibiotics given on day 1 of CDAD treatment.
¶$p < 0.05$.
**$p < 0.001$.

## Interpretation

Few studies have measured the population incidence of CDAD. In Boston it was found to be only 7.7 per 100 000 during 1988–1990.[7] In New Mexico the incidence during 1993–1997 was 14.8 per 100 000, reaching 38.5 per 100 000 among people aged 65–74 years and 98.9 per 100 000 among those aged 75 years or more.[8] In Sweden the incidence of CDAD in 1995 was 58 per 100 000 inhabitants, reaching 121 per 100 000 among adults aged 60–79 and 315 per 100 000 among those 80–89.[9] The incidence of 866.5 per 100 000 that we documented in 2003 among adults aged 65 years or more is several times higher and represented a major epidemic among elderly people, most of which was hospital-acquired, with important consequences.

Recent reports suggesting that CDAD might be evolving into a more severe disease included small numbers of patients and did not adjust for age or other confounding factors.[10,11] Our series documented convincingly an increase in the case-fatality rate of CDAD over the study period, even if some patients died of other causes. The cytotoxicity assay has an excellent specificity, but its sensitivity is only about 82%.[12] Our case definition might thus have overestimated the proportion of cases that were complicated, because patients with milder CDAD who had a negative assay result could be less likely to undergo endoscopy. We found no evidence, however, that use of endoscopy changed over time: in 1991, 3.1% (6/194) of cases were diagnosed by endoscopy, as compared with 2.5% (10/396) in 2003. The change in testing policy in 1996, which resulted in fewer cases being diagnosed in children, might have resulted in a slight bias upwards in the measures of disease severity in subsequent years.

Several factors may have contributed to the emergence of this epidemic. First, following health care reforms promoting ambulatory care, the mean age of patients admitted to hospital in Quebec increased considerably during the last decade, as did the proportion with numerous comorbidities.[13] Whether because of a less effective immune response against *C. difficile* toxins or because of more frequent administration of antibiotics for the treatment of community- or hospital-acquired infections, elderly people are especially susceptible to *C. difficile* infection.[3,14,15] Second, decades of insufficient capital investment in Quebec hospitals have resulted in conditions of hygiene that facilitate the transmission of pathogens such as *C. difficile*. Third, our epidemiological evidence suggests that a more virulent strain might be involved; it seems plausible that it might be more transmissible as well.

Vancomycin is recommended by some for the initial treatment of severe cases of CDAD,[2,4,16,17] with little supporting evidence. Other recent reviews recommend either complete avoidance of vancomycin or its use as a second-line agent in patients with metronidazole failure,[3,5,6,18–21] to

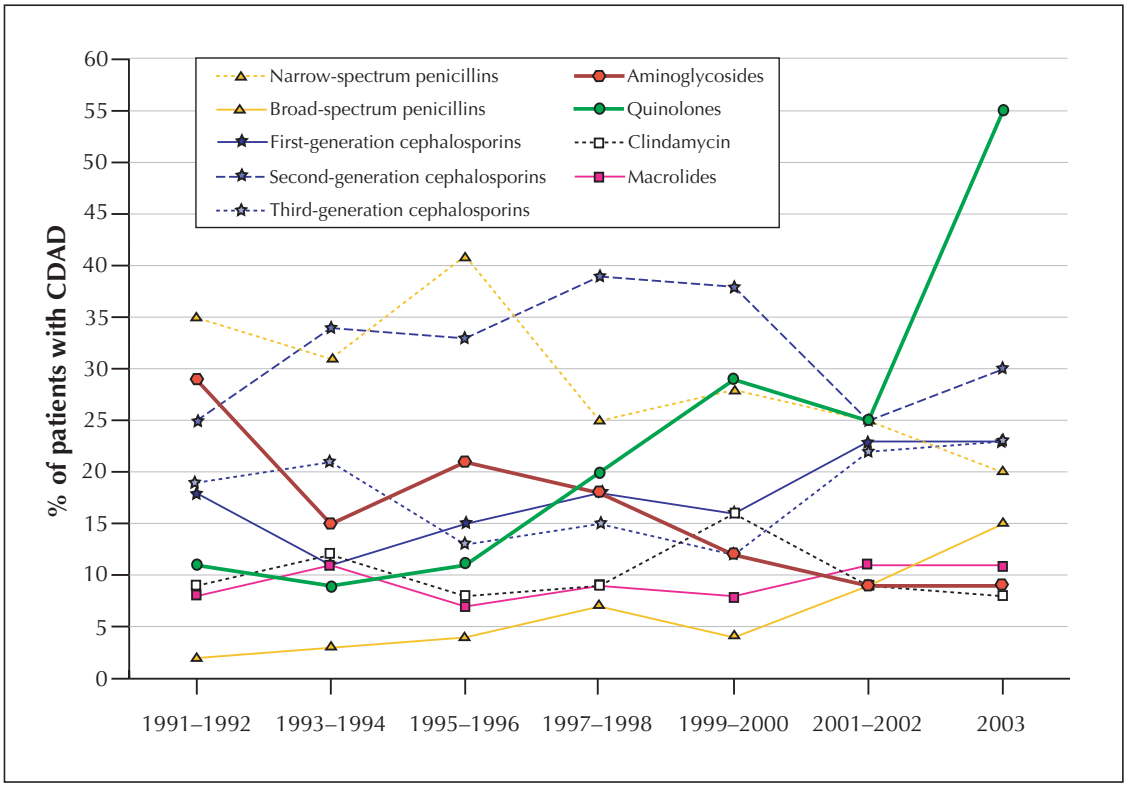


**Fig. 2: Proportions of patients with CDAD by class of antibiotic received in the 2 months preceding the diagnosis of CDAD, 1991–2003.**

avoid promoting the emergence of vancomycin-resistant bacteria. Early studies that claimed equivalence of metronidazole and vancomycin were underpowered.[22–25] A large observational study defined failure as the persistence of diarrhea on day 7, instead of harder end points such as complications of CDAD.[26] In our institution, the proportion of CDAD patients given vancomycin as the initial treatment decreased from 17% in the early 1990s to 3% in 2003. After adjustment for confounding factors, CDAD treated initially with metronidazole progressed to complicated CDAD as frequently as cases left untreated, in contrast to the more favourable outcome with initial vancomycin treatment. This finding should be interpreted cautiously: additional differences in the severity of CDAD upon initiation of treatment (residual confounding) may have been present.

Changes over time in the distribution of antibiotics to some extent reflected variations in the use of these drugs, for instance the replacement of aminoglycosides by quinolones. However, the measures of risk per 1000 patient-days of antibiotic use in our hospital showed that second- and third-generation cephalosporins carried a disproportionate risk of inducing CDAD, as has been observed elsewhere.[27–29] The high risk associated with macrolides reflected their frequent use with third-generation cephalosporins for the treatment of pneumonia. The risk associated with quinolones increased markedly, whereas that associated with β-lactam/β-lactamase inhibitors was similar to that of narrow-spectrum penicillins. A recent report suggested that gatifloxacin might carry a high risk of CDAD;[30] this drug is not on our formulary, and instead the quinolones used during the study period were ciprofloxacin and levofloxacin. We found little evidence that changes in prescribing of antibiotics influenced the dynamics of CDAD during the study period, as shown in Table 3: the use of quinolones and third-generation cephalosporins increased modestly, whereas the use of second-generation cephalosporins decreased. Paradoxically, the use of β-lactam/β-lactamase inhibitors increased markedly.

Leukocytosis and a high creatinine level were strong and independent factors associated with an increased risk of complicated CDAD. This may have been because they reflect different processes, the former being a marker of severe colon inflammation and the latter, the severity of diarrhea. These simple biological markers could be used to select patients who need more aggressive therapy. Given the observational nature of our study, the association between initial treatment with vancomycin and a more favourable outcome needs to be interpreted prudently. Ideally, a randomized controlled trial should address this issue. At our institution, measures to limit the use of second- and third-generation cephalosporins have been implemented and infection control procedures strengthened, with limited impact so far. Future guidelines for the treatment of common infections should take into consideration the risk of inducing CDAD.

This article has been peer reviewed.

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**Table 3: Incidence of hospital-acquired CDAD per 1000 patient-days of use of various classes of antibiotics among all inpatients at the Centre hospitalier universitaire de Sherbrooke**

| | Period; incidence per 1000 patient-days of antibiotic use* | | |
|---|---|---|---|
| Antibiotic class | 1999–2000 | 2001–2002 | 2003 |
| Narrow-spectrum penicillins | 1.4 (28/19908) | 1.2 (25/20597) | 4.9 (53/10751) |
| β-lactam/β-lactamase inhibitors | 1.0 (7/7267) | 1.3 (17/13419) | 5.0 (46/9194) |
| Cephalosporins | | | |
| First-generation | 2.3 (30/12779) | 2.6 (35/13633) | 8.8 (74/8412) |
| Second-generation | 3.9 (55/13984) | 2.9 (36/12224) | 16.3 (92/5639) |
| Third-generation | 2.7 (18/6786) | 4.6 (34/7390) | 19.5 (72/3687) |
| Carbapenems | 2.7 (7/2553) | 6.7 (15/2248) | 7.4 (9/1209) |
| Aminoglycosides | 2.4 (21/8673) | 2.2 (18/8230) | 6.5 (28/4283) |
| Quinolones | 1.6 (48/29693) | 1.2 (36/29375) | 9.9 (161/16293) |
| Clindamycin | 4.9 (19/3861) | 3.1 (11/3508) | 11.7 (22/1880) |
| Macrolides | 1.9 (5/2625) | 4.4 (12/2715) | 20.0 (33/1649) |
| Metronidazole | 2.0 (20/10092) | 1.8 (19/10696) | 5.0 (39/7745) |
| Vancomycin | 2.5 (9/3658) | 2.4 (10/4137) | 5.2 (20/3853) |
| Cotrimoxazole | 0.2 (8/51706) | 0.2 (13/54077) | 0.5 (11/20287) |

*Calculated from numbers in parentheses: the numerator represents the number of patients with hospital-acquired CDAD who received a given class of antibiotic during the 2 months before diagnosis, and the denominator represents the total number of patient-days that this class of antibiotic was used among all inpatients.

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# Effect of procalcitonin-guided treatment on antibiotic use and outcome in lower respiratory tract infections: cluster-randomised, single-blinded intervention trial

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## Summary

**Background** Lower respiratory tract infections are often treated with antibiotics without evidence of clinically relevant bacterial disease. Serum calcitonin precursor concentrations, including procalcitonin, are raised in bacterial infections. We aimed to assess a procalcitonin-based therapeutic strategy to reduce antibiotic use in lower respiratory tract infections with a new rapid and sensitive assay.

**Methods** 243 patients admitted with suspected lower respiratory tract infections were randomly assigned standard care (standard group; n=119) or procalcitonin-guided treatment (procalcitonin group; n=124). On the basis of serum procalcitonin concentrations, use of antibiotics was more or less discouraged (<0·1 μg/L or <0·25 μg/L) or encouraged (⩾0·5 μg/L or ⩾0·25 μg/L), respectively. Re-evaluation was possible after 6–24 h in both groups. Primary endpoint was use of antibiotics and analysis was by intention to treat.

**Findings** Final diagnoses were pneumonia (n=87; 36%), acute exacerbation of chronic obstructive pulmonary disease (60; 25%), acute bronchitis (59; 24%), asthma (13; 5%), and other respiratory affections (24; 10%). Serological evidence of viral infection was recorded in 141 of 175 tested patients (81%). Bacterial cultures were positive from sputum in 51 (21%) and from blood in 16 (7%). In the procalcitonin group, the adjusted relative risk of antibiotic exposure was 0·49 (95% CI 0·44–0·55; p<0·0001) compared with the standard group. Antibiotic use was significantly reduced in all diagnostic subgroups. Clinical and laboratory outcome was similar in both groups and favourable in 235 (97%).

**Interpretation** Procalcitonin guidance substantially reduced antibiotic use in lower respiratory tract infections. Withholding antimicrobial treatment did not compromise outcome. In view of the current overuse of antimicrobial therapy in often self-limiting acute respiratory tract infections, treatment based on procalcitonin measurement could have important clinical and financial implications.

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## Introduction

Lower respiratory tract infections—ie, acute bronchitis, acute exacerbations of chronic obstructive pulmonary disease (COPD) or asthma, and pneumonia—account for almost 10% of the worldwide burden of morbidity and mortality.[1] As much as 75% of all antibiotic doses are prescribed for acute respiratory-tract infections, despite their mainly viral cause.[1] This inappropriate use of antibiotics is believed to be a main cause of the spread of antibiotic-resistant bacteria.[2,3] Thus, reduction of the excess use of antibiotics is essential to combat the increase of antibiotic-resistant microorganisms.[4,5]

To limit antibiotic use, rapid and accurate differentiation of clinically relevant bacterial lower respiratory tract infections from other—mostly viral—causes is pivotal. After obtaining a patient's medical history, physical examination, laboratory tests, and chest radiograph, the clinician is often left with diagnostic uncertainty, because signs and symptoms of bacterial and viral infections widely overlap.[6,7] For example, bacteria can be isolated from sputum in up to 50% of patients with acute exacerbations of COPD, but whether this finding represents colonisation or infection is controversial.[8,9] The absence of specific markers of clinically relevant bacterial infections contributes to the overuse of antibiotics in lower respiratory tract infections, especially in elderly patients with coexisting illnesses.

Circulating amounts of calcitonin precursors, including procalcitonin, are raised in severe bacterial infections, but remain fairly low in viral infections and non-specific inflammatory diseases.[10,11] Findings of many clinical studies have established the superior diagnostic accuracy of procalcitonin in severe infections compared with other markers,[12] albeit that the assay used had a limited functional assay sensitivity of 0·3–0·5 μg/L. Since subtle elevations of circulating procalcitonin are not detected, this assay is not accurate for diagnosis of early or localised infections.[13–15] An improved rapid assay with a functional assay sensitivity of 0·06 μg/L has become available.[16] We aimed to assess the capability of this sensitive procalcitonin assay to identify bacterial lower respiratory tract infections needing antimicrobial treatment.

## Methods

### Patients

This study was a prospective, cluster-randomised, controlled, single-blinded intervention trial comparing routine use of antimicrobial therapy with procalcitonin-guided antimicrobial treatment for lower respiratory tract infections. We assessed for eligibility patients who presented from Dec 16, 2002, until April 13, 2003, with cough, dyspnoea, or both at the medical emergency department of the University Hospital in Basel, Switzerland—a 784-bed academic tertiary care hospital. The criterion for inclusion in the study was a suspected lower respiratory tract infection as the main diagnosis, as

defined below. We excluded severely immunocompromised patients, ie, with HIV infection and a CD4 count less than 200 cells per mL, neutropenic patients, and stem-cell transplant recipients; those with cystic fibrosis or active tuberculosis; and individuals with nosocomial pneumonia.

We randomly assigned eligible patients either standard antimicrobial therapy (standard group) or procalcitonin-guided antimicrobial treatment (procalcitonin group) according to a computer-generated weekwise-randomisation scheme. Thereafter, we prospectively followed up patients during admission or after hospital discharge, respectively. The study was approved by the local ethics committee for human studies at our institution and written informed consent was obtained from all participants. The authors held and analysed all data.

### Procedures

Patients were examined in the emergency room by a resident in internal medicine supervised by a senior registrar, in parallel to the routine treatment of other patients. Assessment included complete history, physical examination, measurement of body temperature, blood sampling for haematological analysis and blood chemistry, including C-reactive protein, and chest radiography. Sputum and blood collection for microbiological culture, blood gases, spirometry, bronchoscopy with bronchoalveolar lavage, and consultation of an infectious disease specialist and pulmonary care specialist were undertaken as needed in both groups.

All patients filled in a visual analogue scale—20 cm wide and ranging from 0% (feeling very ill) to 100% (feeling completely healthy)—and a quality-of-life questionnaire on admission and at follow-up. The quality-of-life questionnaire was used to measure changes in health-related quality of life for patients with respiratory illnesses.[17]

Diagnostic procedures, therapeutic regimen, and final decision to initiate antimicrobial treatment were, in all cases, left to the discretion of the treating doctor. In the procalcitonin group, all doctors had to specify their intention to prescribe antibiotics before they became aware of the serum procalcitonin concentration, referred to as antibiotic prescription foreseen. Thereafter, in this group, we advised the doctor to follow the antibiotic treatment algorithm based on the procalcitonin value.[11–13,18] We judged a serum procalcitonin concentration of 0·1 μg/L or less to indicate absence of bacterial infection, and use of antibiotics was strongly discouraged. This measure was also used in the presence of impaired pulmonary reserve in acute exacerbations of COPD.[13] A procalcitonin value of 0·1–0·25 μg/L was regarded as an indication that bacterial infection was unlikely, and we discouraged use of antibiotics. We deemed serum procalcitonin between 0·25 and 0·5 μg/L to indicate a possible bacterial infection, and the treating doctor was advised to initiate antimicrobial treatment. A procalcitonin value of 0·5 μg/L or greater was judged suggestive of the presence of bacterial infection, and we strongly recommended antibiotic treatment.[11] For patients on antimicrobial therapy at the time of admission, we recommended discontinuation of antibiotics if procalcitonin concentrations were less than 0·25 μg/L. In both groups, re-evaluation 6–24 h after admission was possible in patients in whom antibiotics were withheld, including clinical and laboratory work-up and remeasurement of serum procalcitonin values in the procalcitonin group.

We used a newly developed time-resolved amplified cryptate emission (TRACE) technology assay (Kryptor PCT, BRAHMS, Hennigsdorf, Germany). This assay is based on a sheep polyclonal antibody against calcitonin and a monoclonal antibody against katacalcin, which bind to the calcitonin and katacalcin sequence of calcitonin precursor molecules. The assay has a strikingly improved functional assay sensitivity of 0·06 μg/L—ie, three to five fold above normal mean values.[19] Assay time is 19 min, and in the clinical setting, results can be obtained within 1 h with 20–50 μL of plasma or serum.[16]

We classified community-acquired pneumonia as presence of a new infiltrate on chest radiograph accompanied by one, or several, acquired acute respiratory symptoms and signs: cough; sputum production; dyspnoea; fever greater than 38·0°C; auscultatory findings of abnormal breath sounds and rales; leucocytosis greater than $10^{10}$ cells per L; or

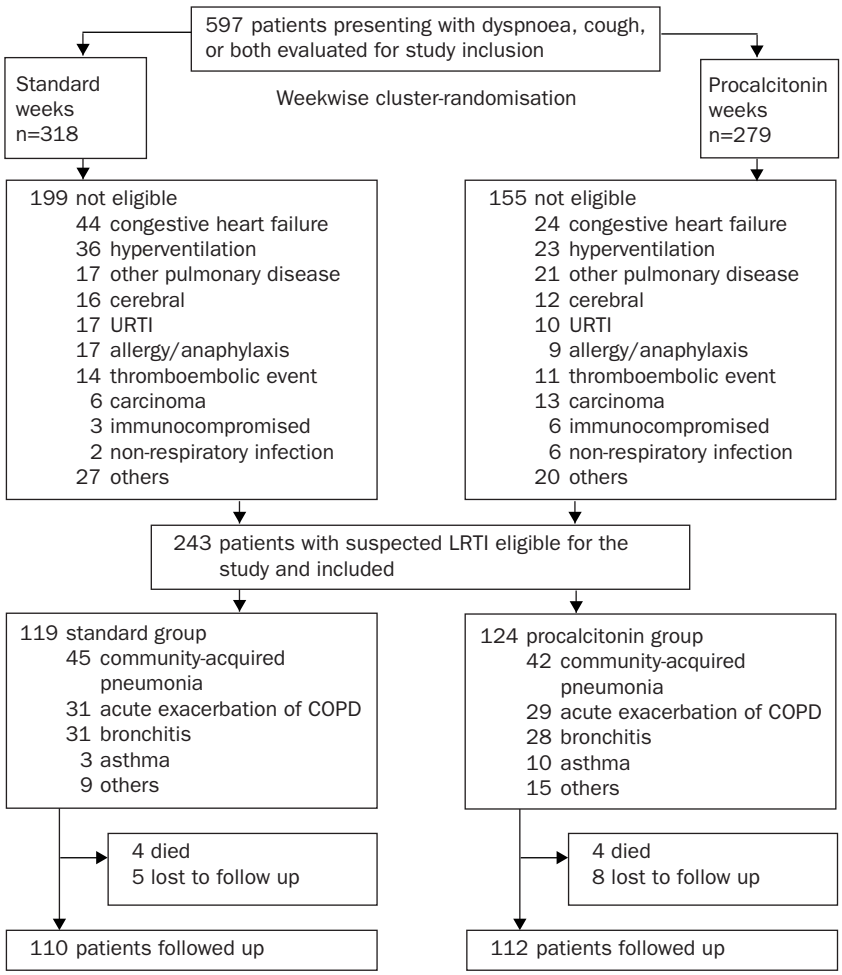


Figure 1: **Trial profile**
URTI=upper respiratory tract infection. LRTI=lower respiratory tract infection.

leucopenia less than $4\times10^9$ cells per L; in the absence of a hospital stay within 14 days before admission.[20,21] We used the pneumonia severity index to estimate severity of pneumonia.[6] We defined COPD according to the global initiative for chronic obstructive lung disease guidelines (http://www.goldcopd.com) as an $FEV_1$/FVC ratio (forced expiratory volume in 1 s/functional vital capacity) less than 70%, with severity categorised into mild ⩾80% of predicted), moderate (⩾50% to <80%), severe (⩾30% to <50%), and very severe (<30%). Severity of acute exacerbations of COPD was defined as proposed.[9] We classified acute bronchitis as acute-onset cough of 2–14 days with or without sputum production in the absence of an underlying lung disease or focal chest signs and infiltrates on chest radiograph, respectively.[7] We defined asthma as episodic symptoms of airflow obstruction, which are at least partly reversible, as assessed by lung-function tests.[22]

We recorded microorganisms if we detected them in sputum, blood cultures, or both, excluding mouth flora. We applied Murray's criteria for validation of the quality of sputum samples. We detected *Legionella pneumophila* antigen either in urine (Legionella now Binax) or by culture or PCR in bronchoalveolar lavage fluid, or by both these methods. We recorded *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* by PCR in bronchoalveolar lavage fluid.[23] We did real-time PCR with a light cycler. We studied serum samples for the presence of antibodies against adenovirus, influenza A, influenza B, parainfluenza virus type 1–3, respiratory syncytial virus, coxsackie B5, cytomegalovirus, Epstein-Barr virus, herpes simplex virus type 1, *M pneumoniae,* and varicella zoster virus, with commercially available, sensitive enzyme immunoassays for IgG and IgM (Orgenium, Turku, Finland). In this kit, IgM and IgG antibody amounts for each specific pathogen are determined separately. We defined detection of IgM above established values, seroconversion of IgG between the acute and convalescence serum samples, high amounts of IgG, or a combination of these factors as serological evidence of acute viral infections, according to the manufacturer's instructions (http://www.orgenium.com).

To assess outcome, a follow-up visit was foreseen 10–14 days after inclusion. Furthermore, in patients with acute exacerbations of COPD, we assessed rate of re-exacerbations and number of readmissions and emergency visits to a doctor by telephone follow-up after 4–6 months.

Our primary endpoint was use of antibiotics. We measured the rate of antibiotic prescriptions in percentage and patient-days and we calculated the relative risk of antibiotic exposure, in patients with lower respiratory tract infections and acute exacerbations of COPD. We calculated the costs of all antimicrobial

| | Standard group (n=119) | Procalcitonin group (n=124) |
|---|---|---|
| **Age (mean [SD], years)** | 65·3 (17·3) | 62·8 (19·8) |
| **Men** | 61 (51%) | 67 (54%) |
| **Smoking status** | | |
| Current smoker | 35 (29%) | 27 (22%) |
| Pack-years history in smokers (mean [SD]) | 40·0 (26·0) | 41·4 (25·0) |
| **Antibiotic pretreatment** | 21 (18%) | 28 (23%) |
| **Coexisting illnesses** | | |
| Coronary artery disease | 32 (27%) | 27 (22%) |
| Congestive heart failure | 7 (6%) | 11 (9%) |
| Peripheral vascular disease | 9 (8%) | 10 (8%) |
| Cerebrovascular disease | 5 (4%) | 4 (3%) |
| Renal dysfunction | 18 (15%) | 22 (18%) |
| Liver dysfunction | 6 (5%) | 6 (5%) |
| Diabetes mellitus | 17 (14%) | 15 (12%) |
| **Symptoms** | | |
| Cough | 111 (93%) | 116 (94%) |
| Sputum | 88 (74%) | 81 (65%) |
| Productive sputum (yellow or green) | 51 (43%) | 54 (44%) |
| Dyspnoea | 78 (66%) | 85 (69%) |
| **Signs** | | |
| Rales | 60 (50%) | 63 (51%) |
| Wheezing | 57 (48%) | 50 (40%) |
| **Chest radiograph infiltrate** | 46 (39%) | 48 (39%) |
| **Additional diagnostic tests** | | |
| Bronchoscopy and bronchoalveolar lavage | 21 (18%) | 22 (18%) |
| Spirometry | 103 (87%) | 109 (88%) |
| Blood cultures | 79 (66%) | 84 (68%) |
| Sputum cultures | 73 (61%) | 87 (70%) |
| Blood gases | 66 (55%) | 59 (48%) |
| Infectious disease specialist consultations | 26 (22%) | 19 (15%) |
| Pulmologist consultations | 17 (14%) | 16 (13%) |
| **Final diagnosis** | | |
| Community-acquired pneumonia | 45 (38%) | 42 (34%) |
| Pneumonia severity index (mean [SD]) | 84·9 (28·9) | 84·3 (41·3) |
| Acute exacerbation of COPD | 31 (26%) | 29 (23%) |
| Acute bronchitis | 31 (26%) | 28 (23%) |
| Acute exacerbation of asthma | 3 (3%) | 10 (8%) |
| Others | 9 (8%) | 15 (12%) |

Data are mean (SD) or number of patients (%). Because of rounding, % might not equal 100.

Table 1: **Baseline characteristics of 243 patients randomly allocated either standard therapy or procalcitonin-guided treatment**

| | Standard group (n=31) | Procalcitonin group (n=29) |
|---|---|---|
| **Age (mean [SD], years)** | 70·1 (7·0) | 71·0 (9·5) |
| **Men** | 18 (58%) | 17 (59%) |
| **Smoking status** | | |
| Current smoker (%) | 14 (45%) | 9 (31%) |
| Pack-years history in smokers (mean [SD]) | 51·9 (26·9) | 48·7 (22·7) |
| **Severity of COPD** | | |
| Very severe | 7 (23%) | 5 (17%) |
| Severe | 15 (48%) | 16 (55%) |
| Moderate | 8 (26%) | 7 (24%) |
| Mild | 1 (3%) | 1 (3%) |
| Baseline $FEV_1$ (mean [SD], L) | 1·1 (0·4) | 1·1 (0·5) |
| Baseline $FEV_1$ % predicted (mean [SD]) | 44·3 (18·8) | 45·2 (15·8) |
| $FEV_1$/FVC ratio (mean [SD]) | 46·2 (14·0) | 44·3 (10·5) |
| **Blood gases*** | | |
| $PaO_2$ (mm Hg) | 60·2 (13·3) | 62·7 (10·5) |
| $PaCO_2$ (mm Hg) | 42·4 (8·9) | 41·2 (8·6) |
| pH | 7·4 (0·06) | 7·4 (0·04) |
| **Symptoms of exacerbation** | | |
| Increasing dyspnoea | 29 (94%) | 26 (90%) |
| Increasing cough | 28 (90%) | 29 (100%) |
| Increasing sputum production | 26 (84%) | 27 (93%) |
| Increasing sputum purulence | 15 (48%) | 19 (66%) |
| **Severity of exacerbation** | | |
| Type 1 | 15 (48%) | 17 (59%) |
| Type 2 | 10 (32%) | 9 (31%) |
| Type 3 | 6 (19%) | 3 (10%) |

Data are mean (SD) or number of patients (%). *Often measured under oxygen administration.

Table 2: **Baseline characteristics of 60 patients with acute exacerbations of COPD randomly allocated either standard therapy or procalcitonin-guided treatment**

| | Standard group (n=119) | | Procalcitonin group (n=124) | | Total |
|---|---|---|---|---|---|
| | Antibiotic-treated (n=99) | No antibiotics (n=20) | Antibiotic-treated (n=55) | No antibiotics (n=69) | |
| **Community-acquired pneumonia** | 45 | – | 38 | 4 | 87 |
| Sputum/bronchoalveolar lavage cultures obtained | 36 | – | 29 | 4 | 69 (79%) |
| Positive | 12 | – | 6 | 1 | 19 (22%) |
| Blood cultures obtained | 41 | – | 38 | 3 | 82 (94%) |
| Positive | 9 | – | 6 | – | 15 (17%) |
| Serologies obtained | 36 | – | 24 | 2 | 62 (71%) |
| Positive | 28 | – | 22 | 1 | 51 (59%) |
| **Acute exacerbation of COPD** | 27 | 4 | 11 | 18 | 60 |
| Sputum/bronchoalveolar lavage cultures obtained | 16 | 3 | 11 | 17 | 47 (78%) |
| Positive | 10 | – | 6 | 11 | 27 (45%) |
| Blood cultures obtained | 14 | – | 9 | 11 | 34 (57%) |
| Positive | – | – | – | – | – |
| Serologies obtained | 19 | 3 | 8 | 13 | 43 (72%) |
| Positive | 14 | 3 | 8 | 6 | 31 (52%) |
| **Bronchitis** | 16 | 15 | 4 | 24 | 59 |
| Sputum/bronchoalveolar lavage cultures obtained | 7 | 4 | 2 | 12 | 25 (42%) |
| Positive | 1 | – | 1 | 2 | 4 (7%) |
| Blood cultures obtained | 9 | 5 | 3 | 7 | 24 (41%) |
| Positive | – | – | – | – | – |
| Serologies obtained | 9 | 11 | 4 | 18 | 42 (71%) |
| Positive | 5 | 9 | 3 | 17 | 34 (58%) |
| **Asthma** | 2 | 1 | – | 10 | 13 |
| Sputum/bronchoalveolar lavage cultures obtained | 1 | – | – | 4 | 5 (39%) |
| Positive | – | – | – | – | – |
| Blood cultures obtained | 2 | – | – | 4 | 6 (46%) |
| Positive | – | – | – | – | – |
| Serologies obtained | 2 | – | – | 8 | 10 (77%) |
| Positive | 2 | – | – | 7 | 9 (69%) |
| **Others** | 9 | – | 2 | 13 | 24 |
| Sputum/bronchoalveolar lavage cultures obtained | 6 | – | 1 | 7 | 14 (58%) |
| Positive | 1 | – | – | – | 1 (4%) |
| Blood cultures obtained | 8 | – | 2 | 7 | 17 (71%) |
| Positive | 1 | – | – | – | 1 (4%) |
| Serologies obtained | 8 | – | 1 | 9 | 18 (75%) |
| Positive | 7 | – | 1 | 8 | 16 (67%) |

Data are number of patients (%).

Table 3: **Microbiology results in different diagnostic subgroups**

agents by adding the prices of all prescribed systemic antimicrobial agents related to treatment of lower respiratory tract infections. We used published average wholesale prices in Switzerland, with an exchange rate of CHF1·34 per US$1.

Additional endpoints were clinical and laboratory outcome—ie, quality-of-life indices, temperature, leucocytes, plasma C-reactive protein and serum procalcitonin concentrations, frequency and length of admission, need for stay in the intensive care unit, death in patients with lower respiratory tract infections, and rate of re-exacerbation and readmission of patients with acute exacerbations of COPD after 6 months.

### Statistical analysis

We expressed discrete variables as counts (%) and continuous variables as mean (SD), unless stated otherwise. The endpoints were predefined and analysed on an intention-to-treat basis. We designed the trial to enrol 105 patients with completed follow-up in each group. This number gave the study 95% power to detect a 30% reduction in antibiotic exposure. Assumptions included use of a two-tailed test, a 5% level of significance, and an SD of 6 days in both groups. Power analysis was done with Primer for Windows, version 4 (McGraw-Hill, New York, NY, USA). We analysed comparability of the standard group and procalcitonin group by $\chi^2$ test, two sample $t$ test, or Mann-Whitney $U$ test, as appropriate. We did analyses with Statistica for Windows version 6 (StatSoft, Tulsa, OK, USA). We used logistic regression to assess the relation between antibiotic prescriptions and age. To adjust for potential clustering effects, we applied the generalised estimating equations approach with STATA version 7.0 (Stata, College Station, TX, USA).

### Role of the funding source

The sponsors of this investigator-initiated project had no role in study design; in collection, analysis, or interpretation of data; in writing of the report; or in the decision to submit the paper for publication.

## Results

Of 4119 patients presenting at the emergency department, 597 (14%) had dyspnoea, cough, or both as main symptoms and were screened for the study. Of these, 243 (41%) were eligible and included (figure 1). Baseline characteristics were similar in both treatment groups, overall (table 1) and in a subgroup of 60 patients with acute exacerbations of COPD (table 2). The group classified as others consisted of 24 patients in whom lower respiratory tract infection was diagnosed on admission by the treating doctor but further evaluation indicated another diagnosis: congestive heart failure (n=5), pulmonary embolism (4), lung cancer (2), mesothelioma (1), lymphangiosis carcinomatosa (1), pleural effusion (2), sarcoidosis (2), usual interstitial pneumonia (2), Dressler's syndrome (1), aspiration pneumonitis (3) and urinary-tract infection with septicaemia (1).

| | Standard group (n=119) | | | | | | Procalcitonin group (n=124) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Antibiotic-treated (n=99) | | | No antibiotics (n=20) | | | Antibiotic-treated (n=55) | | | No antibiotics (n=69) | | |
| | SP | BAL | BC | SP | BAL | BC | SP | BAL | BC | SP | BAL | BC |
| **Community-acquired pneumonia (n=87)** | – | – | – | – | – | – | – | – | – | – | – | – |
| *S pneumoniae* | 8 | 1 | 7 | – | – | – | 2 | – | 4 | – | 1 | – |
| *H influenzae* | 1 | – | – | – | – | – | – | 1 | – | – | – | – |
| *Moraxella catarrhalis* | 2 | – | – | – | – | – | – | – | – | – | – | – |
| *Staphlococcus aureus* | – | 1 | – | – | – | – | – | – | – | – | – | – |
| coag neg Staphylococcus | – | 1 | – | – | – | – | – | – | – | – | – | – |
| *Streptococcus milleri* | – | 1 | – | – | – | – | – | – | – | – | – | – |
| Enterococcus | – | – | – | – | – | – | 1 | – | – | – | – | – |
| *Pseudomonas* spp | 3 | – | 1 | – | – | – | 1 | – | – | – | – | – |
| *Klebsiella* spp | 1 | 1 | – | – | – | – | – | 1 | 1 | – | – | – |
| *Escherichia coli* | – | – | 1 | – | – | – | – | – | 1 | – | – | – |
| Enterobacteriaceae | 1 | – | – | – | – | – | – | – | – | – | – | – |
| *M pneumoniae* | – | 1 | – | – | – | – | – | – | – | – | – | – |
| **Acute exacerbation of COPD (n=60)** | | | | | | | | | | | | |
| *S pneumoniae* | 2 | 1 | – | – | – | – | – | – | – | 1 | – | – |
| *H influenzae* | 1 | – | – | – | – | – | 1 | 1 | – | 5 | 1 | – |
| *S aureus* | – | – | – | – | – | – | – | – | – | 1 | – | – |
| *Pseudomonas* spp | – | – | – | – | – | – | – | 5 | – | 2 | – | – |
| *E coli* | 1 | – | – | – | – | – | – | – | – | – | – | – |
| Enterobacteriaceae | 3 | – | – | – | – | – | – | 3 | – | 3 | – | – |
| *Morganella morganii* | – | – | – | – | – | – | – | 1 | – | – | – | – |
| *Proteus vulgaris* | – | – | – | – | – | – | – | – | – | 1 | – | – |
| **Bronchitis (n=59)** | – | – | – | – | – | – | – | – | – | – | – | – |
| *S pneumoniae* | 1 | – | – | – | – | – | – | – | – | – | – | – |
| *M catarrhalis* | – | – | – | – | – | – | – | 1 | – | 2 | – | – |
| **Others (n=24)** | | | | | | | | | | | | |
| *H influenzae* | 1 | – | – | – | – | – | – | – | – | – | – | – |
| *E coli* | – | – | 1 | – | – | – | – | – | – | – | – | – |

SP=sputum. BAL=bronchoalveolar lavage. BC=blood culture. No growth was reported in cultures taken from patients with asthma.

Table 4: **Bacteria grown from sputum, bronchoalveolar lavage, and blood cultures in different subgroups**

Overall, bacterial cultures were grown from sputum, bronchoalveolar lavage fluid, or both in 51 patients (21%) and from blood in 16 (7%). A similar proportion of microorganisms could be cultured in both groups: in sputum, bronchoalveolar lavage fluid, or both, 24 (20%) and 27 (22%) microorganisms could be grown in the standard and procalcitonin groups, respectively, and in blood, ten (8%) and six (5%) could be grown (table 3). Table 4 shows a detailed analysis of bacterial growth from sputum, bronchoalveolar lavage fluid, and blood cultures in the different diagnostic groups. The most frequently isolated bacteria were *Streptococcus pneumoniae*, *Haemophilus influenzae*, enterobacteriaceae, and *Pseudomonas* spp. One patient with community-acquired pneumonia in the procalcitonin group without antibiotic treatment showed growth of *S pneumoniae* in bronchoalveolar lavage fluid. In this individual, despite a procalcitonin concentration of 0·31 μg/L, the doctor in charge decided to withhold antibiotics, and the patient was discharged from the hospital in good condition. The highest number of positive sputum cultures was reported in patients with acute exacerbations of COPD. In those in the procalcitonin group with acute exacerbations of COPD, the proportion of positive sputum cultures was similar for patients with and without antibiotic treatment. No patients had a positive *L pneumophilia* urine test, and this microorganism was not detected in bronchoalveolar lavage fluid in those who underwent bronchoscopy.

Serological evidence of acute infection was reported in 141 of 175 tested patients (81%; table 3). IgM

| | Standard group (n=119) | | Procalcitonin group (n=124) | | p* |
|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | |
| Quality-of-life score (mean [SD]) | 39·3 (13·2) | 22·9 (15·1) | 41·3 (14·3) | 21·9 (14·7) | 0·60 |
| Visual analogue scale (mean [SD], %) | 43·1 (21·0) | 64·1 (21·5) | 42·5 (20·4) | 65·1 (21·8) | 0·78 |
| Body temperature (mean [SD], °C) | 37·7 (1·1) | 37·0 (0·4) | 37·8 (1·0) | 36·9 (0·3) | 0·06 |
| White-blood-cell count (mean [SD], $\times 10^9$/L) | 12·4 (6·7) | 10·3 (5·1) | 11·7 (6·5) | 9·7 (4·4) | 0·26 |
| C-reactive protein (mean [SD], mg/L) | 97·8 (106·1) | 25·8 (43·7) | 82·8 (93·9) | 18·2 (33·3) | 0·24 |
| Procalcitonin (mean [SD], μg/L) | 1·6 (4·2) | 0·12 (0·2) | 1·6 (7·7) | 0·12 (0·4) | 0·10 |
| Admitted | 88 (74%) | | 101 (81%) | | 0·16 |
| Number of days admitted (mean [SD]) | 11·2 (10·6) | | 10·7 (8·9) | | 0·89 |
| Need for stay in intensive care unit | 6 (5%) | | 5 (4%) | | 0·71 |
| Died | 4 (3%) | | 4 (3%) | | 0·95 |
| Follow-up | 110 (92%) | | 112 (90%) | | 0·56 |
| Follow-up of survivors | 110/115 (96%) | | 112/120 (93%) | | 0·44 |
| Antibiotic prescription foreseen | 99 (83%) | | 99 (80%) | | 0·50 |
| Antibiotics prescribed | 99 (83%) | | 55 (44%) | | <0·0001 |
| Duration of antibiotic treatment (mean [SD], days) | 12·8 (5·5) | | 10·9 (3·6) | | 0·03 |
| Antibiotic use per 1000 days of follow-up (mean [SD]) | 661 (398) | | 332 (433) | | <0·0001 |
| Antibiotic costs per patient (mean [SD], US$) | 202·5 (250·6) | | 96·3 (172·8) | | <0·0001 |

Data are mean (SD) or number of patients (%).*If initial and final data are given, p values denote comparisons between final data of patients in the standard group and final data in the procalcitonin group.

Table 5: **Clinical outcome in all patients with lower respiratory tract infections according to treatment algorithm**

| | Standard group (n=31) | | Procalcitonin group (n=29) | | p* |
|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | |
| Quality-of-life score (mean [SD]) | 45·3 (11·4) | 25·8 (13·7) | 46·1 (15·2) | 27·9 (15·7) | 0·85 |
| Visual analogue scale (mean [SD], %) | 33·8 (15·7) | 55·7 (19·4) | 40·7 (14·2) | 58·8 (17·5) | 0·53 |
| Body temperature (mean [SD], °C) | 37·3 (0·7) | 37·0 (0·4) | 37·5 (0·8) | 36·9 (0·3) | 0·59 |
| White blood cell count (mean [SD], $\times 10^9$/L) | 10·8 (3·5) | 10·5 (3·4) | 11·8 (5·6) | 12·0 (4·1) | 0·18 |
| C-reactive protein (mean [SD], mg/L) | 37·7 (35·4) | 19·1 (34·3) | 55·8 (56·6) | 21·9 (34·2) | 0·42 |
| Procalcitonin (mean [SD], μg/L) | 0·14 (0·26) | 0·13 (0·2) | 0·16 (0·20) | 0·07 (0·1) | 0·61 |
| Admitted | 25 (81%) | | 27 (93%) | | 0·06 |
| Number of days admitted (mean [SD]) | 10·8 (7·0) | | 13·7 (7·3) | | 0·25 |
| Need for stay in intensive care unit | 1 (3%) | | 1 (3%) | | 0·96 |
| Died | 1 (3%) | | 1 (3%) | | 0·96 |
| Follow-up | 30 (97%) | | 28 (97%) | | 0·96 |
| Readmission after study (mean [SD]) | 0·4 (0·8) | | 0·5 (0·8) | | 0·46 |
| Rate of exacerbations after study (mean [SD]) | 0·6 (0·9) | | 0·8 (1·2) | | 0·65 |
| Antibiotic prescription foreseen | 27 (87%) | | 25 (86%) | | 0·92 |
| Antibiotics prescribed | 27 (87%) | | 11 (38%) | | 0·001 |
| Duration of antibiotic treatment (mean [SD], days) | 9·1 (2·8) | | 8·7 (2·1) | | 0·47 |
| Antibiotic use per 1000 days of follow-up (mean [SD]) | 682 (369) | | 269 (414) | | 0·0001 |
| Antibiotic costs per patient (mean [SD], US$) | 101·4 (75·9) | | 64·7 (105·4) | | 0·01 |

Data are mean (SD) or number of patients (%). *If initial and final data are given, p values denote comparisons between final data of patients in the standard group and final data in the procalcitonin group.

Table 6: **Clinical outcome in subgroup with acute exacerbations of COPD**

concentrations were raised in 121 patients. Multiple viral infections were noted in 46 of 175 (26·3%) patients. Parainfluenza virus type 3 (n=44), influenza B (37), adenovirus (29), parainfluenza virus type 1 (19), and respiratory syncytial virus (18) were the most frequent viral infections. Serological evidence of *M pneumoniae* infection was present in three cases.

Outcome after a mean of 13·0 days (SD 5·4) was similar in both groups (table 5). Four deaths in the standard group were due to sepsis (2), myocardial infarction (1), and an unknown cause after discharge (1). Both patients dying with septicaemia had pulmonary comorbidities (lung cancer, lung fibrosis). None of the four deaths in the procalcitonin group was due to delayed or withheld antimicrobial treatment—two were due to myocardial infarction (one with advanced lung cancer and pneumonia), one acute renal failure, and one sepsis (despite immediate and appropriate antimicrobial therapy based on raised procalcitonin concentrations).

During the study, one patient with acute exacerbation of COPD in each group died (table 6). During 5·3 months of follow-up, seven additional patients in this subgroup died, four in the procalcitonin group and three in the standard group: myocardial infarction (2), unknown cause (2), renal failure (1), gastrointestinal bleeding (1), and suicide (1). Re-evaluation of 50 of the 51 surviving patients after mean of 5·3 months (SD 1·1) verified a similar long-term outcome in both groups (table 6).

The rate of antibiotic prescriptions foreseen by the treating doctor was similar in both treatment groups. By contrast, in the procalcitonin group, the proportion of patients with lower respiratory tract infections who received antibiotics was reduced by 47% compared with the standard group (p<0·0001, table 5). In patients with acute exacerbations of COPD the reduction was 56% (p=0·0001, table 6). The relative risk of antibiotic exposure in patients with lower respiratory tract infections in the procalcitonin group was 0·39 (95% CI 0·36–0·42, p<0·0001) and the absolute risk reduction was 50% (95% CI 47–53, p<0·0001). After adjusting for potential confounding factors and possible cluster-effects, the relative risk of antibiotic exposure in the procalcitonin group was 0·49 (0·44–0·55, p<0·0001). Antibiotics prescribed included amoxicillin-clavulanate (standard group 74 [44%], procalcitonin group 34 [38%]), clarithromycin (34 [20%], 19 [21%]), ceftriaxone (22 [13%], 11 [12%]), and others (25%; 39 [23%], 25 [28%]). In the procalcitonin group, mean antimicrobial costs per patient were reduced by 52% in all patients with lower respiratory tract infections (p<0·0001, table 5) and by 36% in those with acute exacerbations of COPD (p=0·012, table 6). Outpatients in whom antibiotics were initially withheld on the emergency room, but given a few days later by the family doctor, were counted as given.

According to the study protocol, the doctor in charge was allowed to overrule the procalcitonin algorithm. Antibiotics were given in nine patients (7·3%) when procalcitonin was less than 0·1 μg/L and in 13 (10·4%) when it was less than 0·25 μg/L, five of whom had community-acquired pneumonia (one with a pulmonary abscess), six acute exacerbations of COPD, and two other disorders (one with pulmonary embolism and one with lymphoma). A re-evaluation 6–24 h after admission was done in both groups in 39 (32%) of 122 patients in whom antibiotics were initially withheld (standard group 16 [37%], procalcitonin group 23 [29%]). In the procalcitonin group, ten received antibiotics thereafter—five because of increasing serum procalcitonin concentrations and five because of the decision of the doctor in charge.

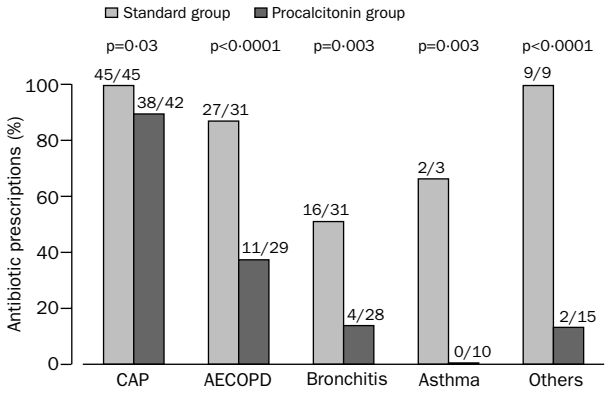


Figure 2: **Antibiotic prescriptions in different subgroups of lower respiratory tract infection comparing standard group and procalcitonin group**

CAP=community-acquired pneumonia. AECOPD=acute exacerbations of COPD.

Antibiotic use could be significantly reduced in all diagnostic subgroups (figure 2). In most patients with community-acquired pneumonia, circulating concentrations of procalcitonin were strikingly raised (standard group 3·9 μg/L [SD 6·2]; procalcitonin group 4·6 μg/L [12·9], p=0·29). In the procalcitonin group, in 10% of patients with this diagnosis (four of 42), antibiotics were withheld on the basis of low serum procalcitonin amounts; all had a favourable outcome, with hospital stays of 1–18 days. On admission, 13 (44·8%) and five (17·2%) patients with acute exacerbations of COPD had serum procalcitonin concentrations of greater than 0·1 μg/L and greater than 0·25 μg/L, respectively.

In the standard group, the odds of being treated with antibiotics increased by 6·5% with every additional year of age (95% CI 3·4–9·8, p<0·0001). Conversely, in the procalcitonin group, no such age relation could be found (95% CI –1·2 to 2·4, p=0·53).

## Discussion

We have shown that procalcitonin guidance substantially and safely reduced antibiotic overuse in patients with lower respiratory tract infections: the risk of antibiotic exposure was reduced by 50%, which equated to 39 fewer antibiotic courses per 100 patients with lower respiratory tract infections. Importantly, withholding antibiotic treatment was safe and did not compromise clinical and laboratory outcome.

Most respiratory-tract infections are due to viral infections.[24] Accordingly, serological evidence of viral infection was reported in almost four-fifths of assessed cases. IgM was positive in most of these individuals, showing the acute nature of viral infection. With less sensitive assays, lower rates of viral infections than ours have been reported, namely for pneumonia.[25–27] Multiple viral infections arose in about a quarter of patients, which compares well with data from seroepidemiological studies.[28] Parainfluenza, influenza, and adenovirus infections accounted for most infections: thus, in most patients in our study, common cold or influenza preceded admission. By damaging the respiratory epithelium, respiratory viruses facilitate secondary bacterial infection of the airways.[29] To separate viral from bacterial infection on the basis of clinical evaluation, serological findings, and bacterial culture is difficult. Based on our data, low serum procalcitonin concentrations of less than 0·1 μg/L to less than 0·25 μg/L can identify patients without clinically relevant bacterial infections; in these individuals antimicrobial therapy can be safely withheld.

Pneumonia is defined as inflammation of the pulmonary parenchyma, which is sometimes caused by a bacterial agent, and in bacterial pneumonia has strikingly raised procalcitonin concentrations.[11,18] Antimicrobial therapy must be promptly initiated because a delay of more than 8 h is associated with increased mortality.[30] Unfortunately, bacteria are usually identified in fewer than half of cases, and positive viral serological findings do not rule out complicating bacterial infection. In the clinical context of community-acquired pneumonia, the primary value of procalcitonin is not reduction of antibiotic prescription, but to facilitate the differential diagnosis of new or progressing infiltrates. If a patient shows new infiltrates on chest radiograph in the presence of acute respiratory symptoms, and procalcitonin concentrations are low, clinicians should seek another diagnosis to bacterial pneumonia (eg, pulmonary embolism, tumour, viral pneumonia). Accordingly, procalcitonin guidance could strikingly lower the number of antibiotic courses in patients with infiltrates on chest radiograph unrelated to pneumonia.

Only a quarter of patients with acute exacerbations of COPD are estimated to benefit from the addition of antibiotics to treatment.[9] The appearance of new strains and persistence of bacterial infection may contribute to this disorder and disease progression, respectively.[31] Most patients with this diagnosis in our study had positive sputum culture results. In the procalcitonin group, this rate was similar in patients in whom antibiotics were given or withheld, as was the outcome, which indicates the limited diagnostic use of sputum cultures in acute exacerbations of COPD. Most patients in whom the procalcitonin-guided treatment algorithm was overruled were in the subgroup with acute exacerbations of COPD. Whether these patients indeed profited from antibiotic therapy remains to be seen. Nevertheless, since individuals with COPD have an impaired pulmonary reserve, and infection might be locally restricted, a procalcitonin concentration of less than 0·1 μg/L as a cutoff to withhold antibiotics is advisable in patients with acute exacerbations with severe disease.

In acute exacerbations of asthma, as far as we know no scientific evidence exists for antibiotic use, and in acute bronchitis, vitamin C used as placebo is as effective as antimicrobial treatment.[17] Accordingly, in these two subgroups of lower respiratory tract infections, antibiotics were only rarely used with procalcitonin guidance. Older patients with these infections are more often treated with antibiotics, because of higher morbidity and mortality especially in the presence of coexisting illnesses.[32] Importantly, this age-related overuse of antibiotic prescriptions could be safely prevented in the procalcitonin group.

Because of the single-blind study design, the treating doctor was aware of the patient's treatment group assignment, which introduced a certain amount of bias. Before the doctor knew the serum procalcitonin amount, a similar proportion of doctors intended to prescribe antimicrobial therapy in both groups. Thus, the reduction of antibiotic prescriptions was not affected by randomisation of a patient into the procalcitonin group per se.

There are large differences in the prescription pattern of antibiotics, overall and for lower respiratory tract infections, between countries and between different health-care providers in the same country.[33,34] However, the area of northern Switzerland has a low antibiotic resistance rate, consistent with a fairly low overall antibiotic use.[35] Thus, for patients admitted to the emergency room of a tertiary care university hospital with severe symptoms of lower respiratory tract infection, the overall antibiotic prescription rate of 83% in the standard group is probably comparable or even lower than in other countries—eg, France or the USA.[36] A reduction of antibiotic use results in fewer side-effects, lower costs, and in the long-term, leads to diminishing drug resistance.[37]

One might argue that antibiotic use in lower respiratory tract infections could also be reduced with C-reactive protein guidance. To the best of our knowledge, no such study has been done in patients with lower respiratory tract infections. Potential caveats limiting reliability of this marker for guidance of antimicrobial treatment are its protracted response with late peak concentrations and suboptimum specificity, especially in patients with severe inflammation and infection.[38]

We must emphasise that the diagnostic accuracy of procalcitonin and its optimum cutoffs are completely dependent on use of a sensitive assay in an appropriate

clinical setting. Procalcitonin is not a substitute for a careful history and physical examination. Yet, as a surrogate marker, it provides important additional information and questions currently used gold standards for the clinical diagnosis of bacterial lower respiratory tract infections. Ideally, an ultrasensitive procalcitonin assay should reliably measure circulating concentrations of this molecule in all healthy individuals. A rapid assay assures that results can be incorporated into clinical decision making, which happened in our study. However, as is the case for all diagnostic tests, a serum procalcitionin concentration must always be evaluated and re-evaluated with proper respect to the clinical context. Circulating procalcitonin can be enhanced in non-infectious disorders, and may remain low even in sepsis.[12,13]

In view of the current overuse of antimicrobial therapy in often self-limiting acute respiratory-tract infections, our findings have important clinical and financial implications.

*Contributors*

B Müller had the idea for the study and directed study design, data analysis and writing of the report. M Christ-Crain drafted the protocol, collected and analysed data, and wrote the report. M M Gencay and P R Huber did laboratory analysis and virological assays. R Bingisser participated in data collection and interpretation of data. D Jaccard-Stolz and M Tamm played a very substantial part in the design of the study, data collection, and writing of the manuscript.

*Conflict of interest statement*

B Müller has served as consultant and received payments from BRAHMS (the manufacturer of procalcitonin assays) to attend meetings related to the trial and for travel expenses, speaking engagements, or research.

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**THE ROYAL COLLEGE OF PATHOLOGISTS**

**Part 2 Examination**

**Thursday 18 November 2004**

**MEDICAL MICROBIOLOGY**

**First Paper**

**Candidates must answer either Question 1 or 2 in Section A and three questions from Section B**

*Time allowed - THREE HOURS*

## Section A

Please answer **either** Question 1 **or** Question 2:

1. What are the main factors determining the prevalence of Methicillin Resistant *Staphylococcus aureus* infection in secondary and tertiary hospitals today? What measures would you recommend at the national level in order to reduce its prevalence?

2. The chief executive officer of your hospital is anxious to introduce new technology to improve turn around and quality. Outline the areas in which you think application of new technology could be implemented and describe your implementation plan for one of these areas.

**Please turn over for Section B**

**Section B**

Please answer three of the following questions:

3. Write short notes on three of the following:

   a) the pathogenesis of *Candida* infections
   b) *Cryptococcus neoformans*
   c) Caspofungin
   d) Dermatophyte infections

4. Write short notes on three of the following:

   a) *Brucella melitensis*
   b) *Plasmodium falciparum*
   c) Pathogenicity mechanisms in *E. coli*
   d) Diagnosis and management of recurrent pinworm infection

5. Write short notes on three of the following:

   a) *Listeria monocytogenes* meningitis
   b) Strategies to prevent group B Streptococcal infection in neonates
   c) Herpes simplex encephalitis
   d) Microbiological investigation of brain abscess

6. Write short notes on three of the following:

   a) Contact tracing in tuberculosis
   b) Norovirus infections in hospitals
   c) The use of glutaraldehyde in hospital disinfection policy
   d) *Salmonella* typing

**THE ROYAL COLLEGE OF PATHOLOGISTS**

**Part 2 Examination**

**Thursday 18 November 2004**

**MEDICAL MICROBIOLOGY**

**Second Paper**

**Candidates must answer all questions in Section A (short answer questions) and two out of three questions in Section B (journal article evaluation questions)**

*Time allowed - THREE HOURS*

## Section A - Short Answer Questions

*Answer all questions.*
*The answers will usually be a single word or a few words.*
*There are no negative marks.*
*When the number of responses is specified* ***DO NOT give additional responses*** *as these will* ***NOT be marked.***

1. A 26 year old Caucasian man who had spent the last ten years in Thailand presented with fever and disseminated pearly umbilicated papular lesions over his face, trunk and limbs. He was found to be HIV positive.

   a) What is the differential diagnosis of these lesions (name four conditions)?

b) A mould producing a red pigment diffusing into the agar was isolated from a biopsy of one of the lesions after 9 days incubation at room temperature. What is this likely to be?

c) How can the diagnosis be confirmed (name three investigations of the organism or the patient)?

d) What are the two drugs of choice for treating this condition?

2. Claire is four years of age and the youngest of two children. Her mother is pregnant again. She developed a generalised itchy discrete red rash which appeared first on her trunk. As they spread from her trunk they developed into small vesicles. She had a few vesicles on the limbs. Claire has no significant previous medical history and is relatively well although she has a temperature of 37.9°C.

a) What is the most likely diagnosis for Claire?

b) What two laboratory tests should be used to confirm this?

c) Claire's 61 year old grandmother develops a similar rash 17 days later. List two potential complications and the most appropriate antiviral drug to be prescribed.

3. Fergal is a 55 year old male ventilation engineer who has been largely healthy throughout his life. He smokes at least 20 cigarettes a day and has done so since he was fourteen. He has a mild "smokers cough" in the morning. He admitted to drinking 20 units of alcohol a week. There was no recent history of foreign travel.
He presented to the accident and emergency unit of his local hospital with shortness of breath and non productive cough. He was confused and disorientated. Clinical examination reveals sparse crackles in the chest which the admitting physician commented could relate to previous smoking. There was no evidence of consolidation. The pulse was 98/min and the blood pressure 120/70. Chest X-ray reveals patchy consolidation throughout the left lung fields. The blood O2 is 8 kPa and the CO2 4.2 kPa. There was no abnormal haematology and the urea and electrolytes were normal. There was a moderate increase (2x normal ) in the AST and ALT. He was deteriorating rapidly.

a) What is your working/the most likely diagnosis in this case?

b) What is the most important urgent bacterial investigation?

c) What antibiotic(s) would you prescribe?

4. Charlie is a five year old boy who has been referred to the GP from the school medical service because of poor vision in his left eye. The child had not noticed any problems with vision and is completely healthy. The family acquired a dog 9 months previously and have one cat which has been a family pet for 10 years. Ophthalmoscopy shows that the posterior chamber is cloudy. The white cell count is normal.

a) What is the most likely diagnosis?

b) How can the diagnosis be confirmed?

c) List two drugs that should be used for treatment

5. A 24 year old female is admitted via the Accident and Emergency department with a temperature of 38.8°C and blood pressure of 90/40mmHg. Her pulse is 120 beats per minute and there is evidence of multiple needle puncture marks on her skin. Her jugular venous pressure is raised and pulsatile. There is a systolic murmur.

a) What is your working diagnosis?

b) What would you expect to be isolated in blood cultures?

c) What is your antibiotic prescription in this case?

d) What will define the prognosis in this patient?

6. FG is an 83 year old female who has recently spent four months in the local district general hospital recovering from an acute lower respiratory tract infection in the Care of the Elderly ward. She had completed a two week course of amoxicillin on the day of discharge from the hospital. She is complaining of watery diarrhoea and feels extremely weak. Clinical examination reveals a temperature of 38°C and a blood pressure of 100/60 mm Hg. Her abdomen is soft. No other members of the family are unwell.

a) What is the most likely diagnosis in this case?

b) What is the most urgent microbiological investigation?

After the correct treatment her condition improves but two weeks later the condition relapses.

c) State your choice of antibiotic and route

d) List the other measures (no more than four) you would take.

7. WG is a 48 year old male of Caribbean origin who has been a regular patient of the rheumatology department where he has been treated for chronic rheumatoid arthritis. Three months ago he had an acute exacerbation of his rheumatoid and treatment with non-steroidal anti-inflammatory agents failed to control it. Ten days ago he was placed on Prednisolone 30 mg daily. Over the last week he has been complaining of a fleeting serpiginous skin rash. His wife remembers that he has complained of this rash some years before. His temperature is 39.4°C and he is hot and dry to the touch. There is no evidence of a skin rash when examined in the accident and emergency room. His blood pressure is 90/40 mm Hg. System examination is normal.

a) What is the most likely diagnosis in this case?

b) What do you expect to isolate from this patient's blood culture?

c) What is (are) the definitive investigation (s) in this case?

d) What drugs are appropriate? (List up to four)

8. SF is a 7 year old female with sickle cell disease. She has had numerous admissions for the management of her severe anaemia. She presents to the accident and emergency room of your district general hospital with a painful left shoulder and upper arm, she is profoundly anaemic.

a) What two infective complications of sickle cell disease may this little girl be suffering from?

The child's temperature is elevated at 38.9°C her CRP is 246 mg/L.

b) What are two most likely organisms that will be isolated from blood culture?

c) List your first choice of antibiotic regimen.

9. A 38 year old female is admitted to the ITU with evidence of shock. One week ago she had been on holiday in France where she had been bitten by mosquitoes and the bite rapidly became infected. A few days afterwards she noted a red track on her leg above the bite and the bite was painful, swollen and inflamed. She felt so unwell that she cut short her holiday to return home.

a) What is the most likely pathological diagnosis?

b) List your management steps.

c) What is the mechanism of this severe illness?

10. A nine year old girl who has recently been visiting relatives in Northern Nigeria presents with profound weakness of the left leg. Two weeks previously, the day after her return from Nigeria she had been unwell with a fever and diarrhoea which had settled soon after.

a) What is the most likely diagnosis?

b) List the steps you would take to protect contacts of this child.

**Section B - Journal article evaluation questions.**

*Answer **two** of the following questions. Your answers should be concise. You should justify your answers by reference to the article where ever possible. In addition, you should include your knowledge of relevant literature when this is available.*

**Question 1**

**Sakoulas G *et al*. Relationship of MIC and Bactericidal Activity to Efficacy of Vancomycin for Treatment of Methicillin-Resistant *Staphylococcus aureus* Bacteremia. *J Clin Microbiol* 2004; 42: 2398-2402.**

a) What type of study is this? Comment on its design and the selection of patients and isolates used.

b) Comment on the significance of the results (as you would expect a consultant microbiologist to be able to interpret the findings).

c) Comment on the implications of this study for the routine clinical microbiology laboratory.

**Question 2**

**Landman D *et al*. Reduction in the incidence of methicillin-resistant *Staphylococcus aureus* and ceftazidime-resistant *Klebsiella pneumoniae* following changes in a hospital antibiotic formulary. *Clin Infect Dis* 1999; 28: 1062–6.**

a) What type of study is this? Comment on its design.

b) Comment on sources of bias in this study.

c) Comment on the way the data were analysed.

d) Faced with these data how would you advise your hospital Drugs & Therapeutics Committee on any action in your Trust?

## Question 3

**Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by Enzyme Immunoassay, Culture, and Three Nucleic Acid Amplification Tests van Dyck et al,. J Clin Microbiol 2001;39:1751-1756**

a) Summarise the composition of the cohorts selected for this study. Comment how the choice of subject has influenced the results and interpretation of the data.

b) The authors have recorded the sensitivity and specificity of the various tests in this paper. What are the strengths and weakness of this approach? Are there alternative measures that could have been used.

c) Summarise briefly the implications of this results of this study for routine diagnosis of *Neisseria gonorrhoeae* infection in a developed country.

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# Relationship of MIC and Bactericidal Activity to Efficacy of Vancomycin for Treatment of Methicillin-Resistant *Staphylococcus aureus* Bacteremia

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**We attempted to find a relationship between the microbiological properties of bloodstream isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and the efficacy of vancomycin in the treatment of bacteremia. Vancomycin susceptibility testing was performed, and bactericidal activity was determined for 30 isolates from 30 different patients with MRSA bacteremia for whom clinical and microbiological outcome data were available. The majority of these patients had been previously enrolled in multicenter prospective studies of MRSA bacteremia refractory to conventional vancomycin therapy. Logistic regression found a statistically significant relationship between treatment success with vancomycin and decreases in both vancomycin MICs (≤0.5 μg/ml versus 1.0 to 2.0 μg/ml; $P = 0.02$) and degree of killing (reduction in $1\log_{10}$ CFU/milliliter) by vancomycin over 72 h of incubation in vitro ($P = 0.03$). For MRSA isolates with vancomycin MICs ≤ 0.5 μg/ml, vancomycin was 55.6% successful in the treatment of bacteremia whereas vancomycin was only 9.5% effective in cases in which vancomycin MICs for MRSA were 1 to 2 μg/ml. Patients with MRSA that was more effectively killed at 72 h by vancomycin in vitro had a higher clinical success rate with vancomycin therapy in the treatment of bacteremia ($\log_{10} < 4.71$ [$n = 9$], 0%; $\log_{10}$ 4.71 to 6.26 [$n = 13$], 23.1%; $\log_{10} > 6.27$ [$n = 8$], 50%). We conclude that a significant risk for vancomycin treatment failure in MRSA bacteremia begins to emerge with increasing vancomycin MICs well within the susceptible range. Elucidating the mechanisms involved in intermediate-level glycopeptide resistance in *S. aureus* should begin by examining bacteria that begin to show changes in vancomycin susceptibility before the development of obvious resistance. Prognostic information for vancomycin treatment outcome in MRSA bacteremia may also be obtained by testing the in vitro bactericidal potency of vancomycin.**

Vancomycin has been the cornerstone of therapy for serious methicillin-resistant *Staphylococcus aureus* (MRSA) infections since the early 1980s, when MRSA emerged as a significant nosocomial pathogen in the United States (2, 11, 25, 32). However, many clinicians believe that the efficacy of vancomycin against MRSA is inferior to that of antistaphylococcal beta-lactams against methicillin-susceptible *S. aureus* (MSSA) infections (11, 14). The basis of this belief comes partly from in vitro data that demonstrate slower bactericidal activity of vancomycin compared to the results seen with antistaphylococcal beta-lactams against *S. aureus* (1, 28) as well as data suggesting slow clinical response (13, 14). In our recent study of evaluating factors associated with clinical failure in 87 patients with MRSA bacteremia, we found evidence of a relationship between vancomycin MIC and clinical vancomycin failure by univariate analysis, although this did not prove to be an independent predictor of failure in multivariate analysis (19). Some view the higher level of mortality seen with MRSA bacteremia compared to the results seen with methicillin-susceptible *S. aureus* bacteremia as another example of the inferiority of vancomycin to beta-lactams (4).

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Although the emergence of glycopeptide-intermediate-level-resistant *S. aureus* (GISA) (8, 9, 29) and, most recently, glycopeptide-resistant *S. aureus* (3) are reasons for concern, these cases are quite rare. Nevertheless, vancomycin treatment failures are not uncommon with MRSA infections despite the organism being fully susceptible (vancomycin MIC ≤ 2 μg/ml) by standard methods of testing and criteria (6, 7, 8, 10, 17, 18, 26). Antimicrobial regimens that provide bactericidal therapy have been demonstrated to be superior to bacteriostatic regimens in the treatment of *S. aureus* bloodstream infections, especially with infective endocarditis (5, 22, 23). We performed this study to determine whether the microbiological properties of clinical MRSA in the presence of vancomycin in vitro correlated with the clinical efficacy of vancomycin in the treatment of bacteremia. We used data available to us through patients that had been enrolled previously in prospective studies for the purposes of receiving alternative therapy for MRSA bacteremia because of either intolerance to or failure of conventional vancomycin therapy.

## MATERIALS AND METHODS

**Clinical isolates.** During the period of July 1998 to November 2001, 87 patients were enrolled in phase III and IV multicenter prospective studies, yielding 122 MRSA isolates from 24 different hospitals across 16 states (19). To further explore the relationship between clinical failure of vancomycin in treatment of MRSA bacteremia and susceptibility to inhibition and killing by vancomycin in

TABLE 1. Patient characteristics of 30 patients with MRSA infections for whom in vitro testing of a clinical isolate was performed

| Characteristic | Value for group[a] | | P |
|---|---|---|---|
| | VAN treatment success[b] | VAN treatment failure | |
| Age in yr (mean ± SD [median]) | 63 ± 15 (62) | 59 ± 17 (60) | 0.5 |
| Sex | | | 0.7 |
| Male | 4 (57) | 9 (39) | |
| Female | 3 (43) | 14 (61) | |
| Located in the ICU | 2 (29) | 10 (43) | 0.7 |
| $Log_{10}$ (CFU/ml) of killing (mean ± SD [median]) | 6.26 ± 1.29 (6.27) | 4.88 ± 1.81 (5.10) | 0.07 |

[a] Data are no. (%) of patients.
[b] VAN, vancomycin.

vitro, a subset of 30 of these isolates from 30 patients was subjected to additional testing in this study. The majority of patients were enrolled in these studies for the purpose of receiving alternative therapy after unsatisfactory response to conventional therapy. Isolates were stored at −70°C until the time of in vitro testing. Vancomycin susceptibility was performed under blinded conditions with Mueller-Hinton II agar (Becton Dickinson, Cockeysville, Md.) and an inoculum of $10^4$ CFU/ml according to recommendations of the NCCLS (20). Growth was evaluated at 24 h at 35°C. Quality control of susceptibility testing was performed using ATCC 33591 (MRSA) reference strains. Bactericidal activity of vancomycin was determined under blinded conditions without knowledge of any clinical outcome. All isolates were susceptible to vancomycin (MIC ≤ 2 μg/ml).

**Case definitions.** Vancomycin treatment failure was defined in patients that received at least 5 days of appropriately dosed vancomycin by serum level monitoring (trough, 10 to 15 μg/ml) when one or more of the following were present in association with persistently positive blood cultures: (i) persistent signs or symptoms of infection (fever, leukocytosis, etc.); (ii) new additional signs or symptoms of infection; or (iii) worsening of signs or symptoms of infection observed at baseline. Serum concentrations of vancomycin were measured by commercially available immunoassay methods employed in clinical laboratories of participating hospitals.

**Vancomycin bactericidal assays.** Overnight (14 to 18 h) cultures of MRSA in brain heart infusion broth (Becton Dickinson, Sparks, Md.) were diluted 1:100 in a 250-ml Erlenmeyer flask to a final volume of 20 ml in fresh brain heart infusion broth containing vancomycin (16 μg/ml), yielding a starting inoculum of approximately $10^7$ to $10^8$ CFU/ml. Flasks were incubated at 35°C with gentle swirling for 30 s once daily. Samples obtained at 0 and 72 h were diluted serially $1{:}10^0$ to $1{:}10^7$, and 25 μl of each dilution was plated in duplicate on sheep blood agar plates to determine counts of viable bacteria. Vancomycin bactericidal activity for each strain was expressed as $\log_{10}$ CFU per milliliter (0 h) − $\log_{10}$ CFU per milliliter (72 h). MRSA ATCC 33591 was used as a control in these experiments. Exposure of this strain to vancomycin (16 μg/ml) in four separate experiments resulted in a mean (± standard deviation) reduction in viable counts at 72 h of 4.57 (± 0.18) $\log_{10}$ CFU/ml. The maximum and minimum repeats were within 0.4 $\log_{10}$ CFU/ml.

**Statistical analysis.** Dichotomous variables were compared using chi-square analysis or Fisher's exact test where appropriate. Continuous variables were analyzed with the Kruskal-Wallis analysis of variance test. We used classification and regression tree modeling (Systat version 10; SPSS Inc., 2000), a form of binary recursive partitioning, to identify breakpoints in $\log_{10}$ CFU of killing/milliliter at 72 h for analysis of vancomycin response. Tree-based models are useful for both classification and regression problems. This type of modeling identifies what dichotomous split on which predictor variable will maximally improve the predictability of the dependent variable. The predictor variable(s) may be a mixture of nominal and/or ordinal scales. The dependent variable may be quantitative or qualitative (i.e., nominal or categorical). The regression trees parallel regression analysis of variance modeling, and the classification trees parallel discriminant analysis. Finally, logistic regression was used to determine whether a relationship existed between vancomycin treatment success and two explanatory variables: vancomycin MIC and $\log_{10}$ CFU of killing/ml at 72 h.

## RESULTS

We analyzed 30 MRSA isolates from 30 patients with MRSA bacteremia to determine their susceptibility or resistance to killing by vancomycin. In vitro testing of these isolates was performed in a blinded fashion without knowledge of any clinical outcomes. Of the patients tested, 23 patients were vancomycin treatment failures and 7 were treated successfully. There were no significant differences in age, sex, and proportion of intensive care unit patients between the two groups (Table 1). We noted marked heterogeneity in the in vitro bactericidal activity of vancomycin against MRSA, with reduction in viable bacteria at 72 h ranging from 0.17 $\log_{10}$ to 8.16 $\log_{10}$ CFU/ml. With patients for whom vancomycin treatment was successful, MRSA demonstrated increased killing in vitro at 72 h (mean, 6.26 $\log_{10}$ CFU/ml) compared to the results seen with those for whom treatment failed (mean, 4.88 $\log_{10}$ CFU/ml). This difference approached but did not achieve statistical significance ($P = 0.07$).

Classification and regression tree modeling identified two breakpoints resulting in three subgroups with respect to the magnitude of vancomycin $\log_{10}$ CFU of killing/ml at 72 h for analysis of vancomycin treatment efficacy: group 1, <4.71; group 2, 4.71 to 6.26; group III, ≥6.27. There were significant differences in the percentages of patients successfully treated with vancomycin in the three groups: 0% for group 1, 23.1% for group 2, and 50% for group 3 (Table 2). There were no statistically significant differences in duration of prior clinical vancomycin exposure prior to obtaining isolates in any of the three groups to account for the differences in killing (Table 2).

We found no significant relationship between the bactericidal activity of vancomycin and the MIC of vancomycin. The values of median vancomycin $\log_{10}$ CFU of killing/ml at 72 h were 5.94 and 5.40 against MRSA isolates for which vancomycin MICs were ≤0.5 μg/ml and 1 to 2 μg/ml, respectively (Table 3).

In a prior study in which we examined the relationship of the accessory gene regulator (*agr*) group II genotype and vanco-

TABLE 2. Rate of vancomycin success and in vitro vancomycin bactericidal activity

| Group | $Log_{10}$ (CFU/ml) of killing | n | % VAN success[a] | No. of days of VAN Rx prior to isolate procedure[b] |
|---|---|---|---|---|
| 1 | <4.71 | 9 | 0.0 | 33 ± 30 (20) |
| 2 | 4.71–6.26 | 13 | 23.1 | 44 ± 79 (20) |
| 3 | ≥6.27 | 8 | 50.0 | 23 ± 27 (15.5) |

[a] Group 1 versus 3, $P = 0.029$; group 1 versus 2 versus 3, $P = 0.05$. VAN, vancomycin.
[b] Data are means ± SD (median); VAN Rx, vancomycin therapy.

TABLE 3. Vancomycin treatment success rates and vancomycin bactericidal activity by sensitivity of the MRSA isolate to vancomycin

| VAN[a] MIC (μg/ml) | *n* | $Log_{10}$ (CFU/ml) of killing (mean ± SD [median]) | $Log_{10}$ (CFU/ml) of killing for group: | | | % VAN success[b] |
|---|---|---|---|---|---|---|
| | | | <4.71 | 4.71–6.26 | ≥6.27 | |
| ≤0.5 (5) | 9 | 4.91 ± 2.26 (5.94) | 3 | 3 | 3 | 55.6 |
| 1.0–2.0 | 21 | 5.32 ± 1.59 (5.40) | 6 | 10 | 5 | 9.5 |

[a] VAN, vancomycin.
[b] $P = 0.01$ (Fisher's exact test).

mycin treatment failure for MRSA bacteremia, we noted that the vancomycin MIC had an impact on treatment failure in univariate analysis (19). However, when multivariate analysis only was used, patient elevated serum creatinine levels and the *agr* group II genotype were significant predictors of vancomycin treatment failure. When a subset of the same clinical isolates was analyzed in the present study, we again noted a relationship between vancomycin treatment failure and elevated vancomycin MIC. We found a significant decrease in vancomycin treatment efficacy of vancomycin for MRSA isolates with vancomycin MIC values of 1 to 2 μg/ml (9.5% clinical success rate) compared to the results seen with isolates with vancomycin MIC values of ≤0.5 μg/ml (55.6% clinical success rate; $P = 0.01$) (Table 3).

Multivariate analysis using logistic regression showed a statistically significant relationship between the increased therapeutic efficacy of vancomycin and both lower vancomycin MIC and increased killing by vancomycin in vitro (Table 4).

## DISCUSSION

The clinical importance of bactericidal therapy in the treatment of most infections remains controversial. However, in treatment of infective endocarditis, experience during the antibiotic era of the past 6 decades suggests that bactericidal activity is an important determinant of clinical outcome. In one study of 20 patients with serious *S. aureus* infections of whom 80% had endocarditis, patients who received bactericidal therapy (99.9% 24-h killing in vitro) demonstrated a more rapid clinical and microbiological cure and showed a significantly lower mortality of 0% compared to 40% mortality in patients who received bacteriostatic therapy (5). In another study of *S. aureus* endocarditis, the addition of gentamicin to nafcillin resulted in more rapid clinical and microbiological response but had no effect on mortality (12).

More recent studies investigating the importance of bactericidal activity in the treatment of *S. aureus* bacteremia in an era in which methicillin resistance has emerged are lacking. Despite the recent introduction of linezolid and quinupristin-dalfopristin for use by clinicians in the treatment of serious infections due to gram-positive organisms, vancomycin remains the treatment of choice for serious MRSA infections. These newer agents are generally bacteriostatic against MRSA. However, tolerance to vancomycin among clinical *S. aureus* isolates has been previously described (15, 21, 24, 30, 31). We wanted to investigate whether decreased killing by vancomycin in vitro translated to any clinical effect of treatment.

TABLE 4. Multivariate analysis of factors associated with vancomycin treatment success

| Factor | OR (95% CI)[a] | *P* |
|---|---|---|
| Increased VAN killing[b] | 10.73 (1.24–92.95) | 0.031 |
| Decreased VAN MIC[c] | 35.46 (1.76–715.95) | 0.020 |

[a] OR, odds ratio; CI, confidence interval.
[b] The odds ratio for increased vancomycin killing represents the incremental increased chance of treatment success between the groups identified by regression tree modeling ($log_{10}$ [CFU/ml] of killing at 72 h: group 1, <4.71; group 2, 4.71 to 6.26; group 3, ≥6.27). VAN, vancomycin.
[c] The odds ratio for decreased vancomycin MIC represents the increased chance of success for treatment of MRSA infection with vancomycin MIC ≤0.5 μg/ml versus MIC 1.0 to 2.0 μg/ml.

We investigated the relationship between the degree of vancomycin bactericidal activity in vitro and clinical treatment outcome in MRSA bacteremia. We found a positive correlation with the bactericidal activity of vancomycin in vitro and clinical success in the treatment of bacteremia. MRSA that demonstrated less than 4.7 $log_{10}$ killing at 72 h showed 0% successful treatment with vancomycin, whereas isolates that demonstrated ≥6.27 $log_{10}$ killing at 72 h demonstrated 50% success.

We found no differences in mortality on the basis of vancomycin bactericidal activity, perhaps a result of the fact that isolates used in this study were obtained from compassionate use studies of linezolid and quinupristin-dalfopristin in which patients who failed vancomycin treatment were offered alternative treatment. This also explains the high rate of vancomycin failure in the sample studied.

Classification and regression tree modeling were used to determine breakpoints of bactericidal killing to provide a fair distribution of the sample into three groups. We chose 72 h as our time point to assess killing in vitro to maximally differentiate isolate characteristics in the setting of the relatively slow bactericidal activity of vancomycin. The killing assay used here did not allow for enough discrimination between isolates for earlier endpoints. Although vancomycin is very stable, longer periods of incubation would have raised questions as to the possibility of breakdown of the compound. Our unpublished observations suggest that failure to achieve a 99.9% bactericidal endpoint after 24 h of incubation with vancomycin at 16 μg/ml is not uncommon in testing recent isolates of MRSA. This concentration is close to what is typically achieved in patients undergoing vancomycin therapy (16). The relatively high-level inoculum was chosen to maximally differentiate killing among the different strains.

In vitro comparisons of clinical isolates are very difficult to control for prior exposure of the organism to antibiotic. We tried to control for vancomycin exposure of the tested organism within the patient from whom it was isolated to address

confounding by vancomycin exposure in the individual patient. However, we were unable to control for any exposure that the organism may have had to vancomycin in any previously colonized or infected patient with the same strain.

Despite the fact that hospital-associated MRSA isolates in the United States share a fairly homogeneous genetic background (27), our findings demonstrated significant heterogeneity in the bactericidal activity of vancomycin against MRSA, with 72-h killing ranging from 0.17 to 8.16 $\log_{10}$. This may be a possible explanation for the variable success seen in patients with MRSA bacteremia treated with vancomycin (8).

The vancomycin killing assay used in this investigation allowed us to demonstrate that increased bactericidal activity of vancomycin against MRSA may predict a higher probability of clinical success in the treatment of MRSA bacteremia. However, it should be pointed out that employing such methods in the clinical laboratory is impractical because they are too time consuming. In addition, although our data confirm a relationship between susceptibility to inhibition and killing by vancomycin in vitro and response to vancomycin treatment of MRSA bacteremia, the utility of these methods for testing individual isolates is doubtful. For example, we identified individual MRSA bloodstream isolates from patients who failed to respond to vancomycin therapy which demonstrated greater killing than other isolates from patients who were treated successfully. However, our findings of decreased efficacy of vancomycin in MRSA bacteremia with isolates for which vancomycin MICs were 1 to 2 μg/ml suggests that useful clinical information may be extrapolated from a clinical microbiology susceptibility report. While decreased vancomycin efficacy may be expected in isolates for which vancomycin MICs are higher, as observed for hetero-GISA and GISA (MIC, 4 to 16 μg/ml) (8), we found it noteworthy that the efficacy of vancomycin began to decline for isolates with vancomycin MICs that lie well within the susceptible range. Despite the high rate of treatment failure with vancomycin in this study, we found no MRSA isolates for which the vancomycin MIC was >2 μg/ml. While we used agar dilution susceptibility testing in this study, there is no reason to suspect that similar findings could not be anticipated with susceptibility reports from automated systems employed in many hospital laboratories. However, although MIC measurements are readily obtained in the clinical microbiology laboratory, a more precise measurement of how useful MIC data can be to clinicians and confirmation of these findings would require a larger study of patients in a group perhaps not weighted as heavily towards vancomycin treatment failures.

It is important to point out that because our collection of isolates was drawn from a group of patients of whom most had failed vancomycin therapy, it would be inappropriate to extrapolate from the results of this paper a quantitative prediction of vancomycin treatment failure rate with each incremental increase in vancomycin MIC.

A multivariate analysis using logistic regression showed a statistically significant relationship between increased vancomycin efficacy and both decreased vancomycin MIC (≤0.5 μg/ml) and increased vancomycin killing. A sample size of only 30 patients was sufficient to demonstrate the statistical significance of these variables. However, the small sample size resulted in extremely large confidence intervals when calculating odds ratios; therefore, the magnitudes of the odds ratios are difficult to interpret. A more quantitative analysis would require a larger study.

A final extrapolation of these findings is this: for investigators to fully elucidate the multiple-step genetic pathways involved in the development of intermediate-level glycopeptide resistance in *S. aureus*, studies should begin with the analysis of isolates that show subtle microbiological changes in the presence of glycopeptides before the development of overt resistance.

In summary, we demonstrated that vancomycin-susceptible clinical MRSA isolates demonstrate considerable heterogeneity in vitro with respect to vancomycin MIC and vancomycin killing. These differences appear to affect the clinical efficacy of vancomycin and the probability of successful treatment of MRSA bacteremia.

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# Reduction in the Incidence of Methicillin-Resistant *Staphylococcus aureus* and Ceftazidime-Resistant *Klebsiella pneumoniae* Following Changes in a Hospital Antibiotic Formulary

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**In 1995, changes in our hospital formulary were made to limit an outbreak of vancomycin-resistant enterococci and resulted in decreased usage of cephalosporins, imipenem, clindamycin, and vancomycin and increased usage of $\beta$-lactam/$\beta$-lactamase-inhibitor antibiotics. In this report, the effect of this formulary change on other resistant pathogens is described. Following the formulary change, there was a reduction in the monthly number (mean $\pm$ SD) of patients with methicillin-resistant *Staphylococcus aureus* (from 21.9 $\pm$ 8.1 to 17.2 $\pm$ 7.2 patients/1,000 discharges; $P = .03$) and ceftazidime-resistant *Klebsiella pneumoniae* (from 8.6 $\pm$ 4.3 to 5.7 $\pm$ 4.0 patients/1,000 discharges; $P = .02$). However, there was an increase in the number of patients with cultures positive for cefotaxime-resistant *Acinetobacter* species (from 2.4 $\pm$ 2.2 to 5.4 $\pm$ 4.0 patients/1,000 discharges; $P = .02$). Altering an antibiotic formulary may be a possible mechanism to contain the spread of selected resistant pathogens. However, close surveillance is needed to detect the emergence of other resistant pathogens.**

In the past decade, outbreaks of emerging resistant pathogens (e.g., vancomycin-resistant enterococci and Enterobacteriaceae having extended-spectrum $\beta$-lactamases) have been described [1–7], and established pathogens (e.g., methicillin-resistant *Staphylococcus aureus*) have persisted [8–10] and infiltrated the community [9, 10]. Guidelines have emphasized aggressive infection-control measures to limit the spread of resistant bacteria within hospitals [11, 12]. However, it is troubling that many of these outbreaks have occurred in the era of universal precautions, and investigations describing the failure of a variety of infection-control measures have been reported [1–3, 8, 9, 13, 14]. Clearly, new approaches are needed to limit the nosocomial spread of resistant bacteria.

See editorial response by Rice on pages 1067–70.

In 1993, infection-control measures were instituted at our hospital to limit the spread of vancomycin-resistant enterococci. When the failure of these measures was realized [2], we attempted to control the outbreak by changing the hospital formulary. In May 1995, the use of $\beta$-lactam/$\beta$-lactamase-inhibitor antibiotics was emphasized and the use of third-generation cephalosporins, vancomycin, and clindamycin was restricted. The success of this intervention to limit the spread of vancomycin-resistant enterococci and *Clostridium difficile* has been previously reported [15]. In this study, we examined the effect of this intervention on other nosocomial pathogens.

## Methods

The Department of Veterans Affairs Medical Center at Brooklyn is a university-affiliated tertiary care facility. Microbiology records from 1 January 1993 through 30 April 1997 were reviewed to identify all patients whose cultures yielded the following bacteria: methicillin-resistant *S. aureus,* ceftazidime-resistant *Klebsiella pneumoniae,* ceftazidime-resistant *Enterobacter* species, ceftazidime-resistant *Pseudomonas aeruginosa,* ticarcillin-resistant *P. aeruginosa,* and cefotaxime-resistant *Acinetobacter* species. These organisms were selected to assess the impact of formulary changes on the major nosocomial pathogens at our institution. Only patients whose cultures of blood or other sterile body fluids or of wound, respiratory tract, or urinary tract specimens yielding the targeted pathogens were included. The number of new patients each month who had a positive culture was recorded. For each pathogen, patients were included only once. All isolates were identified with standard microbiological methods, and susceptibility testing was performed according to the guidelines of the National Committee for Clinical Laboratory Standards [16]. Of the antipseudomonal penicillins, only ticarcillin was routinely used in susceptibility testing. In 1996, the laboratory tested the susceptibility of selected resistant isolates to piperacillin/tazobactam.

In May 1995, approval by an infectious diseases physician was required prior to the administration of third-generation cephalosporins, clindamycin, and vancomycin. Ampicillin/sul-

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bactam and piperacillin/tazobactam were added to the formulary, and their use was suggested in place of the cephalosporins. In addition, pharmacy records from 1 January 1993 through 31 December 1996 were reviewed to determine the overall antibiotic usage per month. The usage and expenditures for the following antibiotics were reviewed: ampicillin, penicillin, piperacillin, nafcillin, ampicillin/sulbactam, piperacillin/tazobactam, cefazolin, cefoxitin, cefotaxime, ceftazidime, imipenem, ciprofloxacin, vancomycin, clindamycin, metronidazole, amikacin, and gentamicin. To adjust for fluctuating prices, antibiotic costs were calculated with use of 1996 prices.

Infection-control measures directed against vancomycin-resistant enterococci were initiated in April 1993, as previously described [2]. In May 1995, in addition to the changes in the hospital antibiotic formulary, contact precautions were expanded to also include patients with *C. difficile* colitis and diarrhea of unknown etiology [15]. No specific precautions were undertaken for patients whose cultures yielded any of the bacteria studied in this investigation.

*Statistical analysis.* The incidence of each nosocomial pathogen is reported as the number of new patients with a positive culture per 1,000 discharges per month from the medical and surgical services. The usage of antibiotics is reported as grams utilized per month. Results are expressed as mean ± SD. Student's *t* test and $\chi^2$ analysis were used to compare preintervention and postintervention data. Stepwise multiple linear regression analysis was performed to determine any correlation between the number of patients with cultures yielding methicillin-resistant *S. aureus* or ceftazidime-resistant *K. pneumoniae* and the following variables: number of discharges, average length of stay, and the usage of vancomycin, clindamycin, and third-generation cephalosporins. All data were analyzed with use of TRUE EPISTAT software (Epistat Services, Houston). A two-tailed *P* value of $\leq .05$ was considered significant.

## Results

Usage of the three targeted antibiotics (cefotaxime, clindamycin, and vancomycin) significantly decreased following the intervention in May 1995. Monthly usage of cefotaxime fell from 1,432 ± 283 to 164 ± 78 g/mo ($P < .001$), that of clindamycin from 594 ± 167 to 108 ± 62 g/mo ($P < .001$), and that of vancomycin from 588 ± 136 to 313 ± 98 g/mo ($P < .001$). In addition, significant reductions in the use of four antibiotics not targeted by the intervention were observed: cefazolin (from 724 ± 187 to 531 ± 209 g/mo [$P = .002$]), ceftazidime (from 677 ± 197 to 229 ± 117 g/mo [$P < .001$]), imipenem (from 136 ± 57 to 89 ± 47 g/mo [$P = .004$]), and gentamicin (from 43.8 ± 14.1 to 30.6 ± 6.9 g/mo [$P < .001$]). Administration of the two $\beta$-lactamase-inhibitor combination antibiotics dramatically increased: that of ampicillin/sulbactam to 3,326 ± 853 g/mo and that of piperacillin/tazobactam to 1,898 ± 761 g/mo. Total antibiotic costs before and after the intervention did not vary significantly ($29,457 ± $3,866 vs. $28,085 ± $5,749 per month [$P = .3$]).

For the 29 months before the intervention, the monthly incidence of new patients with cultures positive for methicillin-resistant *S. aureus* was 21.9 ± 8.1 per 1,000 discharges (figure 1). For the 23 months following the intervention, this decreased to 17.2 ± 7.2 patients per 1,000 discharges ($P = .03$). The incidence of new patients with ceftazidime-resistant *K. pneumoniae* also decreased, from 8.6 ± 4.3 to 5.7 ± 4.0 per 1,000 discharges ($P = .02$; figure 1). Susceptibility testing of the isolates from the 50 patients identified just prior to the intervention in May 1995 revealed that only 6% were resistant to cefotaxime and cefoxitin, suggesting the presence in most isolates of a $\beta$-lactamase that could be inhibited by sulbactam, tazobactam, and clavulanate. Of the isolates from the 50 patients identified immediately following the intervention, 10% were resistant to cefotaxime and cefoxitin ($P = .07$).

Compared with that in the baseline period, there was no change in the incidence of new patients with ceftazidime-resistant *Enterobacter* species (5.3 ± 3.2 vs. 4.1 ± 2.0 per 1,000 discharges [$P = .10$]) or ceftazidime-resistant *P. aeruginosa* (3.8 ± 2.9 vs. 3.3 ± 2.8 per 1,000 discharges [$P = .5$]). Similarly, there was no difference in the incidence of new patients with ticarcillin-resistant *P. aeruginosa* before and after the intervention (11.4 ± 4.7 vs. 12.2 ± 4.2 [$P = .5$]). However, the incidence of new patients with cultures positive for cefotaxime-resistant *Acinetobacter* species rose significantly (figure 1), from 2.4 ± 2.2 to 5.4 ± 4.0 per 1,000 discharges before and after the intervention, respectively ($P = .02$). Of 25 isolates collected in 1997, all but one were resistant to piperacillin/tazobactam.

The number of hospital discharges from the medical and surgical services averaged 569 ± 50 per month before the intervention. Following the intervention, this decreased to 513 ± 50 per month ($P < .001$). In addition, the average length of stay on the acute care services also decreased, from 15.0 ± 1.5 to 13.2 ± 1.8 days ($P < .001$). Multiple regression analysis revealed that only usage of third-generation cephalosporins was significantly correlated with the number of patients with methicillin-resistant *S. aureus* ($P = .003$) and ceftazidime-resistant *K. pneumoniae* ($P < .001$). The number of new patients requiring contact isolation (those with *C. difficile* or vancomycin-resistant enterococci) also decreased following the intervention, from 10.7 ± 4.7 patients per month to 6.2 ± 4.5 patients per month ($P = .002$).

Review of microbiological records revealed that 61% of 2,447 *S. aureus* isolates collected from 1993 through 1995 were susceptible to methicillin; in 1996, 65% of 678 isolates were susceptible ($P = .04$). For *K. pneumoniae,* 66% of 1,374 isolates received by the microbiology laboratory from 1993 through 1995 were susceptible to ceftazidime, compared with 88% of 336 isolates collected in 1996 ($P < .001$). The percentage of isolates of *P. aeruginosa* susceptible to ceftazidime also increased, from 92% of 1,560 isolates collected from 1993

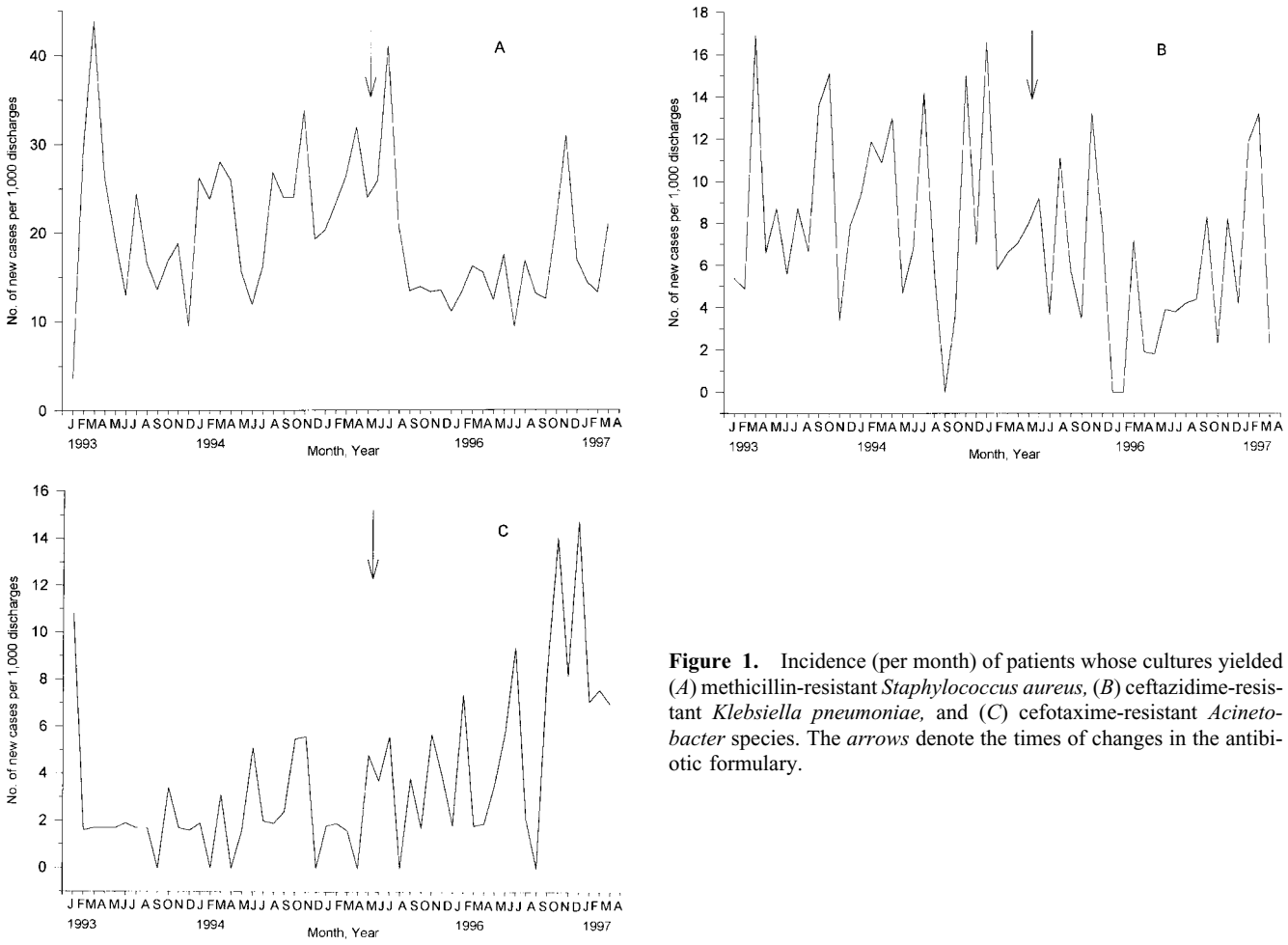


**Figure 1.** Incidence (per month) of patients whose cultures yielded (*A*) methicillin-resistant *Staphylococcus aureus,* (*B*) ceftazidime-resistant *Klebsiella pneumoniae,* and (*C*) cefotaxime-resistant *Acinetobacter* species. The *arrows* denote the times of changes in the antibiotic formulary.

through 1995 to 96% of 438 isolates collected in 1996 ($P = .003$). There was no change in the percentage of *Enterobacter* species isolates susceptible to ceftazidime or in the percentage of *P. aeruginosa* isolates susceptible to ticarcillin. There was a significant decrease in the percentage of *Acinetobacter* species isolates susceptible to cefotaxime, from 51% of 392 isolates collected from 1993 through 1995 to 37% of 155 isolates collected in 1996 ($P = .005$).

## Discussion

There is an increasing trend for nosocomial infections to be caused by more-resistant pathogens [17], and as new antimicrobial agents have been developed, bacteria have acquired new mechanisms of resistance [18]. Although infection-control protocols may help limit the spread of nosocomial pathogens, once introduced into the hospital, pathogens are often difficult to eradicate [1–3, 8–10, 13, 14]. The emergence of a particular pathogen may be enhanced by the selective pressures exerted by the antibiotics used at a hospital. Changing the antibiotic usage may be an effective method for limiting the spread of resistant pathogens.

Methicillin-resistant *S. aureus* is a frequent nosocomial pathogen [19]. We noted a significant decline in the incidence of patients with cultures positive for methicillin-resistant *S. aureus* following a reduction in vancomycin, clindamycin, and cephalosporin usage and an increase in ampicillin/sulbactam and piperacillin-tazobactam usage. Although most methicillin-resistant *S. aureus* isolates are resistant to $\beta$-lactamase-inhibitor combination antibiotics, negating the effect of penicillinase restores much of the activity of ampicillin [20–22]. $\beta$-Lactam/$\beta$-lactamase-inhibitor antibiotics have shown activity in the treatment of experimental endocarditis due to methicillin-resistant *S. aureus* [20–22], although high levels may be required [22, 23]. Therefore, colonization with methicillin-resistant *S. aureus* may be less likely to occur in a patient receiving a $\beta$-lactam/$\beta$-lactamase-inhibitor antibiotic (than in one receiving a cephalosporin). Emphasizing this class of antibiotics may reduce the incidence of infection with this pathogen.

*K. pneumoniae* strains having extended-spectrum $\beta$-lactamases have been isolated with increasing frequency [4], and hospital outbreaks have been described [5–7]. Some of these outbreaks have involved strains with $\beta$-lactamases inhibited

by β-lactamase inhibitors [5–7]. Many of our isolates were susceptible to cefoxitin or cefotaxime, a trait characteristic of *K. pneumoniae* with β-lactamases effectively inhibited by clavulanate, sulbactam, or tazobactam [5, 7, 24–26]. Experimental infections involving such strains have been successfully treated with β-lactam/β-lactamase inhibitors [27], although increasing levels of the inhibitor may be required [28–30].

The decreasing incidence of patients with ceftazidime-resistant *K. pneumoniae* following our intervention is likely due to the ability of the β-lactamase-inhibitor antibiotics to prevent colonization or infection with these pathogens. Similar decreases were not noted for ceftazidime-resistant *Enterobacter* species or *P. aeruginosa,* which are more likely to carry chromosomal β-lactamases unaffected by β-lactamase inhibitors [18, 25, 31].

Unfortunately, a significant increase in resistant *Acinetobacter* isolates was noted following this intervention; virtually all were resistant to β-lactamase-inhibitor antibiotics. Therefore, while our intervention had a significant impact on the number of patients with methicillin-resistant *S. aureus* and ceftazidime-resistant *K. pneumoniae,* close surveillance is needed to detect the emergence of other resistant pathogens.

Other factors may have contributed to the decline in the number of patients with methicillin-resistant *S. aureus* and ceftazidime-resistant *K. pneumoniae.* It is unlikely that this decline was related to infection-control practices, since the number of patients in isolation actually decreased following the intervention. Consistent with a nationwide trend, there has been a significant decrease in the average length of stay at our hospital. Shorter hospital stays may reduce the likelihood that patients will be exposed to nosocomial pathogens. However, despite the shorter length of stay, the number of patients with other ceftazidime-resistant pathogens remained the same (or increased). We found it compelling that the only isolation of methicillin-resistant *S. aureus* and ceftazidime-resistant *K. pneumoniae,* microorganisms inhibited by β-lactamase-inhibitor antibiotics, decreased. Moreover, multiple linear regression analysis supported the observation that the change in the formulary, and not the length of stay, was associated with the number of patients with these two pathogens.

Changes in antibiotic usage have contributed to the control of outbreaks of *C. difficile* [13, 15], vancomycin-resistant enterococci [15], and ceftazidime-resistant *K. pneumoniae* [7, 32]. A recent report noted a significant decline in infections due to resistant *Klebsiella pneumoniae* when third-generation cephalosporin use decreased and imipenem and piperacillin/tazobactam use increased [32]. If surveillance reveals the emergence of a resistant pathogen at a particular hospital, the overall susceptibility trends and antibiotic usage should be reviewed. Altering the antibiotic formulary on the basis of this information may be a possible mechanism to contain the spread of the pathogen.

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# Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by Enzyme Immunoassay, Culture, and Three Nucleic Acid Amplification Tests

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**The purpose of this study was to evaluate and compare three commercially available nucleic acid amplification tests (NAATs) for the detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Roche PCR and Becton Dickinson strand displacement amplification (SDA) were performed on 733 endocervical swab specimens from commercial sex workers. Abbott ligase chain reaction (LCR) was performed on a subset of 396 samples. Endocervical specimens from all women were also tested by culture for *N. gonorrhoeae* and by Syva MicroTrak enzyme immunoassay (EIA) for *C. trachomatis*. A positive *N. gonorrhoeae* result was defined as a positive result by culture or by two NAATs, and a positive *C. trachomatis* result was defined as a positive result by two tests. According to these definitions, the sensitivities and specificities for the subsample of 396 specimens of *N. gonorrhoeae* culture, PCR, SDA, and LCR were 69.8, 95.2, 88.9, and 88.9% and 100, 99.4, 100, and 99.1%, respectively; the sensitivities and specificities of *C. trachomatis* EIA, PCR, SDA, and LCR were 42.0, 98.0, 94.0, and 90.0% and 100, 98.0, 100, and 98.6%, respectively. The performance characteristics of *N. gonorrhoeae* culture, PCR, and SDA and *C. trachomatis* EIA, PCR, and SDA for all 733 specimens were defined without inclusion of LCR results and by discrepant analysis after resolution of discordant *N. gonorrhoeae* PCR results and of discordant *C. trachomatis* EIA and PCR results by LCR testing. The sensitivities of *N. gonorrhoeae* culture, PCR, and SDA before and after LCR resolution were 67.8, 95.7, and 93.9% and 65, 95.8, and 90.0%, respectively. The sensitivities of *C. trachomatis* EIA, PCR, and SDA decreased from 39.4, 100, and 100% to 38.7, 98.7, and 94.7%, respectively. All three NAATs proved to be superior to *N. gonorrhoeae* culture and to *C. trachomatis* EIA. The accuracies of the different NAATs were quite similar. SDA was the only amplification assay with 100% specificity for detection of both *N. gonorrhoeae* and *C. trachomatis* in endocervical specimens.**

*Chlamydia trachomatis* and *Neisseria gonorrhoeae* are two of the most prevalent sexually transmitted pathogens, with high rates of infection among female commercial sex workers in developing countries, a substantial proportion of whom have asymptomatic infections. Traditional laboratory diagnosis of these infections is done by culture for *N. gonorrhoeae* and cell culture or antigen detection for *C. trachomatis*. Recently, nucleic acid amplification tests (NAATs) have become widely used; these tests have shown a greater sensitivity and have improved the ability to detect *C. trachomatis* and *N. gonorrhoeae* infections. Several studies have shown that NAATs are more accurate than the former standard tests for *C. trachomatis* and *N. gonorrhoeae* (2–6, 11–13, 16, 22, 25–28, 30, 33). However, it is known that certain substances in clinical specimens may be associated with amplification inhibition and that NAATs may give false-positive results (7, 9, 16, 20, 21, 29, 31). Currently available commercial *C. trachomatis* and *N. gonorrhoeae* DNA amplification tests include PCR (Roche Molecular Systems, Branchburg, N.J.), the ligase chain reaction (LCR; Abbott Laboratories, Abbott Park, Ill.), and strand displacement amplification (SDA; Becton Dickinson, Sparks, Md.).

A major drawback for comparative studies of different commercialized NAATs for the detection of *N. gonorrhoeae* and *C. trachomatis* in genital swabs is the incompatibility of the various specimen collection and transport systems, each accompanied by particular handling instructions and DNA extraction procedures. To allow a scientifically valid comparison and a correct head-to-head evaluation of different diagnostic assays, all tests should be performed on the same single specimen and the study should include a substantial number of true-positive specimens.

The purpose of this study was to evaluate the performance of SDA, PCR, and LCR in a reference laboratory setting for the detection of *N. gonorrhoeae* and *C. trachomatis* in single endocervical swabs stored and transported in dry tubes. Locally performed *N. gonorrhoeae* culture and *C. trachomatis* enzyme immunoassay (EIA), used for management of patients, were also evaluated.

## MATERIALS AND METHODS

**Study population and clinical specimens.** Between September 1996 and April 2000, a multicenter study on the effectiveness of a vaginal microbicide to prevent human immunodeficiency virus (HIV) infection among female commercial sex workers was conducted in Cotonou, Benin; Durban, South Africa; and Hat Yai, Thailand. After giving written informed consent, women were screened for HIV infection and other sexually transmitted diseases, and HIV-negative women were included in the study and followed up on a monthly basis. Dacron swabs were used to collect endocervical specimens each month for the detection of *N. gonorrhoeae* and *C. trachomatis*. A first swab was used for *N. gonorrhoeae* culture on modified Thayer Martin medium. A second swab was used for *C. trachomatis* EIA antigen detection (MicroTrak; Syva, San Jose, Calif.). A third swab was kept

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TABLE 1. Pattern of *N. gonorrhoeae* test results for 396 endocervical specimens

| Result of: | | | | No. of specimens |
|---|---|---|---|---|
| Culture | PCR | SDA | LCR | |
| + | + | + | + | 38 |
| − | + | + | + | 14 |
| + | − | − | − | 2 |
| + | + | + | − | 2 |
| + | + | − | − | 1 |
| + | − | − | + | 1 |
| − | + | + | − | 2 |
| − | + | − | + | 3 |
| − | + | − | − | 2[a] |
| − | − | − | + | 3[b] |
| − | − | − | − | 328 |

[a] False positive by PCR.
[b] False positive by LCR.

dry in a sterile cryovial at 4°C after collection, stored at −20°C within 5 h, and shipped on dry ice to the Institute of Tropical Medicine, Antwerp, Belgium, for *C. trachomatis-N. gonorrhoeae* coamplification PCR testing (Amplicor; Roche Diagnostic Systems, Branchburg, N.J.). After arrival, swabs were stored at −20°C until testing.

**Sample preparation and processing.** Dry swabs ($n$ = 733) collected consecutively between May 1999 and December 1999 (Cotonou, $n$ = 317; Durban, $n$ = 235; Hat Yai, $n$ = 181) were used for comparison of different NAATs to detect *N. gonorrhoeae* and *C. trachomatis*. The first 396 specimens were tested in parallel by PCR (Amplicor; Roche), SDA (BDProbeTec ET; Becton Dickinson), and LCR (LCx; Abbott). The next 337 specimens were tested by PCR and SDA; *N. gonorrhoeae* culture-negative, PCR-SDA-discordant samples and samples positive by (*C. trachomatis*) EIA, PCR, or SDA only were tested by LCR for *N. gonorrhoeae* or *C. trachomatis*, respectively.

For processing, specimens were thawed and kept at room temperature for 2 h, and 1.2 ml of diluted phosphate-buffered saline (9 parts saline and 1 part phosphate-buffered saline) was added to each vial. Samples were vortexed vigorously at maximum speed for 2 min. After removal of the swabs, four aliquots of 250 μl of sample suspension were pipetted in small conic tubes and used for immediate testing or kept at −20°C for later testing.

For each amplification assay, a 250-μl sample was centrifuged at 12,000 × *g* for 10 min and the pellet was used for DNA extraction, as follows. (i) For PCR, 250 μl of Amplicor lysis buffer was added to the pellet. After vortexing for 30 s and incubation for 15 min at room temperature, the sample was mixed with 250 μl of specimen diluent. After another vortexing, 50 μl of the treated sample was used for the PCR assay following the instructions of the manufacturer. (ii) For SDA, 1 ml of test-specific diluent was added to the pellet. After vortexing for 30 s, the manufacturer's procedure was strictly followed and 100 μl of the treated specimen was used for amplification. (iii) For LCR, 1 ml of test-specific urine resuspension buffer was added to the pellet. After vortexing for 30 s and heating, 100 μl of the treated specimen was used for amplification according to the instructions of the manufacturer. The NAATs were performed blindly by three different technicians. Amplicons were detected according to the different test procedures, and specimens with assay values equal to or greater than the cutoff were considered positive.

**Analysis of results.** Specimens tested in parallel by PCR, SDA, and LCR were considered true positive for *N. gonorrhoeae* if they were positive by culture or by two NAATs and true positive for *C. trachomatis* if they were positive by any two tests (EIA, PCR, SDA, or LCR).

Specimens tested in parallel by PCR and SDA were considered *N. gonorrhoeae* true positive if they were positive by culture or by both amplification assays and *C. trachomatis* true positive if they were positive by any two tests (EIA, PCR, or SDA). For *N. gonorrhoeae* culture-negative, PCR-SDA-discordant samples and for *C. trachomatis* EIA-, PCR-, or SDA-only-positive samples, *N. gonorrhoeae* LCR or *C. trachomatis* LCR tests were performed, and LCR-positive samples were considered true positive in discrepant analysis.

The sensitivity and specificity of culture, PCR, SDA, and LCR for *N. gonorrhoeae* and of EIA, PCR, SDA, and LCR for *C. trachomatis* were calculated for specimens tested in parallel ($n$ = 396) by all three NAATs. The sensitivity and specificity of *N. gonorrhoeae* culture, PCR, and SDA and of *C. trachomatis* EIA, PCR, and SDA were also calculated for all specimens ($n$ = 733) before and after inclusion of supplemental LCR data obtained for samples with discordant results after parallel testing. Ninety-five percent confidence intervals (CIs) were calculated based on the binomial distribution of the observed test results.

## RESULTS

***N. gonorrhoeae*.** All 733 specimens were tested by culture. The first 396 specimens were tested by PCR, SDA, and LCR in parallel, the next 337 specimens were tested by PCR and SDA, and LCR was performed on PCR-positive, SDA-negative, culture-negative samples only. None of the samples was found to be inhibitory when tested for the internal control in PCR or in SDA.

For the first 396 specimens, the pattern of test results is shown in Table 1: 328 specimens were negative by all tests, 63 (15.9%) specimens were *N. gonorrhoeae* positive, and 5 specimens were positive in one test only. Table 2 shows the sensitivities of culture, PCR, SDA, and LCR. The 95% CIs revealed no differences between the three NAATs. Culture was significantly less sensitive than PCR.

For the 337 samples tested by culture, PCR, and SDA, the results of all three tests were identical for 310 (92.0%), while 55 (16.3%) specimens were *N. gonorrhoeae* culture positive or positive by both PCR and SDA. Three culture-negative specimens were PCR positive but SDA negative. Table 2 shows the sensitivities of culture, PCR, and SDA. The 95% CIs revealed no differences between the two NAATs. Culture was significantly less sensitive.

Table 3 shows the combined results of culture, PCR, and SDA for all specimens ($n$ = 733). According to the definitions, 115 samples were *N. gonorrhoeae* positive: 78 (68.2%) cases were detected by culture, and 37 (32.2%) were detected by the combination of PCR and SDA. In addition, 8 samples were PCR positive but negative by culture and by SDA: 5 of these were LCR positive (3 were detected among the first 396 sam-

TABLE 2. Sensitivity and specificity of *N. gonorrhoeae* diagnostic tests

| Specimen group ($n$) | Test | Sensitivity (%) | 95% CI | Specificity (%) | 95% CI |
|---|---|---|---|---|---|
| Tested by 4 techniques (396) | Culture | 44/63 (69.8) | 57.0–80.8 | 333/333 (100) | 98.9–100 |
| | PCR | 60/63 (95.2) | 86.7–99.0 | 331/333 (99.4) | 97.8–99.9 |
| | SDA | 56/63 (88.9) | 78.4–95.4 | 333/333 (100) | 98.9–100 |
| | LCR | 56/63 (88.9) | 78.4–95.4 | 330/333 (99.1) | 97.4–99.8 |
| Tested by 3 techniques (337) | Culture | 34/55 (61.8) | 47.7–74.6 | 282/282 (100) | 98.7–100 |
| | PCR | 53/55 (96.4) | 87.5–99.6 | 279/282 (98.9) | 96.9–99.8 |
| | SDA | 52/55 (94.5) | 84.9–98.9 | 282/282 (100) | 98.7–100 |

TABLE 3. Combined results of culture, PCR, and SDA for all 733 endocervical swabs tested for gonococcal infection

| Result of: | | | No. of specimens |
|---|---|---|---|
| Culture | PCR | SDA | |
| + | + | + | 71 |
| + | − | − | 5 |
| + | + | − | 2 |
| − | + | + | 37 |
| − | + | − | 8[a] |
| − | − | − | 610 |

[a] Five were LCR positive, and three were LCR negative

TABLE 5. Pattern of *C. trachomatis* test results for 396 endocervical specimens

| Result of: | | | | No. of specimens |
|---|---|---|---|---|
| ELISA | PCR | SDA | LCR | |
| + | + | + | + | 19 |
| − | + | + | + | 23 |
| + | + | + | − | 1 |
| + | − | − | + | 1 |
| − | + | + | − | 4 |
| − | + | − | + | 2 |
| − | + | − | − | 7[a] |
| − | − | − | + | 5[b] |
| − | − | − | − | 334 |

[a] False positive by PCR.
[b] False positive by LCR.

ples, and 2 were detected among the 337 specimens, for which LCR was performed on 3 discordant PCR-positive samples only.

By combining all 733 specimens, the performance characteristics of *N. gonorrhoeae* culture, PCR, and SDA can be estimated with exclusion of all LCR data or with inclusion of the LCR results for the 8 PCR-positive discordant samples. Sensitivities and specificities of culture, PCR, and SDA before and after discrepant analysis by PCR are shown in Table 4. Before discrepant analysis the number of *N. gonorrhoeae*-positive samples was 115; sensitivities of culture, PCR, and SDA were 67.8, 95.7, and 93.9%, respectively. After additional testing by LCR of PCR-positive discordant samples, the number of *N. gonorrhoeae*-positive samples increased to 120 and the performance of culture, PCR, and SDA did not change significantly. Culture was significantly less sensitive than either NAAT.

***C. trachomatis*.** All specimens were tested by enzyme-linked immunosorbent assay (ELISA). The first 396 specimens were tested by PCR, SDA, and LCR, and the next 337 specimens were tested by PCR and SDA, with LCR being performed on specimens showing one positive result by either EIA, PCR, or SDA.

For the first 396 specimens, the pattern of test results is shown in Table 5: 50 (12.6%) samples were *C. trachomatis* positive, and there were 7 PCR-only-positive and 5 LCR-only-positive results. Table 6 shows the sensitivities and specificities of ELISA, PCR, SDA, and LCR. ELISA was significantly less sensitive than the NAATs.

For the 337 specimens tested by two NAATs (PCR and SDA), the results of ELISA, PCR, and SDA were identical for 314 (93.2%), while 24 (7.1%) specimens were *C. trachomatis* positive in at least two assays. Table 6 shows the sensitivities of ELISA, PCR, and SDA. The 95% Cls revealed no differences between the two NAATs. ELISA was significantly less sensitive.

The samples positive by one test only were additionally tested by LCR: one ELISA-only-positive sample was positive by LCR, and one out of seven PCR-only-positive samples was positive by LCR.

Table 7 shows the combined results of ELISA, PCR, and SDA for the total of 733 specimens. According to the definitions, 71 samples were *C. trachomatis* positive; all 71 were identified by both PCR and SDA, while ELISA identified 28 positive samples. One sample was positive by ELISA and negative by PCR and SDA but was positive by LCR; 16 samples were PCR positive and negative by ELISA and SDA, and 3 of these were LCR positive.

Because ELISA, PCR, and SDA were performed on all specimens, sensitivities and specificities of the three tests can be estimated with exclusion of all LCR results or with inclusion of the LCR results for ELISA-only-positive and PCR-only-positive samples. The performance characteristics of ELISA, PCR, and SDA before and after discrepant analysis by LCR are shown in Table 8. Before discrepant analysis, the number of *C. trachomatis*-positive samples was 71. After additional testing by LCR of the samples positive by one test only, the total number of *C. trachomatis*-positive samples increased to 75 and the sensitivities of ELISA, PCR, and SDA decreased slightly. The specificity of SDA remained 100%; for ELISA it changed from 99.8 to 100%, and for PCR it changed from 97.6 to 98.0%.

## DISCUSSION

The performance and evaluation of NAATs for the detection of *N. gonorrhoeae* and *C. trachomatis* in genital specimens has been the subject of much study and controversy. We com-

TABLE 4. Performance characteristics of *N. gonorrhoeae* culture, PCR, and SDA before and after resolution of discordant results

| Test | Discrepant analysis[a] | Sensitivity (%) | 95% CI | Specificity (%) | 95% CI |
|---|---|---|---|---|---|
| Culture | A | 78/115 (67.8) | 58.5–76.4 | 618/618 (100) | 99.4–100 |
| | B | 78/120 (65) | 55.8–73.5 | 613/613 (100) | 99.4–100 |
| PCR | A | 110/115 (95.7) | 90.1–98.6 | 610/618 (98.7) | 97.5–99.4 |
| | B | 115/120 (95.8) | 90.5–98.6 | 610/613 (99.5) | 98.6–99.9 |
| SDA | A | 108/115 (93.9) | 87.9–97.5 | 618/618 (100) | 99.4–100 |
| | B | 108/120 (90.0) | 83.2–94.7 | 613/613 (100) | 99.4–100 |

[a] A, before additional testing of discordant results; B, after resolution of discordant positive PCR results by LCR testing.

TABLE 6. Sensitivity and specificity of *C. trachomatis* diagnostic tests

| Specimen group (*n*) | Test | Sensitivity (%) | 95% CI | Specificity (%) | 95% CI |
|---|---|---|---|---|---|
| Tested by 4 techniques (396) | ELISA | 21/50 (42.0) | 28.2–56.8 | 346/346 (100) | 98.9–100 |
| | PCR | 49/50 (98.0) | 89.4–99.9 | 339/346 (98.0) | 95.9–99.2 |
| | SDA | 47/50 (94.0) | 83.5–98.6 | 346/346 (100) | 98.9–100 |
| | LCR | 45/50 (90.0) | 78.2–96.7 | 341/346 (98.6) | 96.7–99.5 |
| Tested by 3 techniques (337) | ELISA | 8/24 (33.3) | 15.6–55.3 | 312/313 (99.7) | 98.2–100 |
| | PCR | 24/24 (100) | 85.8–100 | 306/313 (97.8) | 95.4–99.1 |
| | SDA | 24/24 (100) | 85.8–100 | 313/313 (100) | 98.8–100 |

pared the results of PCR, SDA, and LCR for *N. gonorrhoeae* and *C. trachomatis* as well as those obtained by culture for *N. gonorrhoeae* and by antigen ELISA for *C. trachomatis*.

The specificity of DNA amplification assays can be ensured by retesting initially positive specimens by a different amplification method, as was done in the present study. It is more difficult to evaluate the sensitivity of NAATs (or any other assays) when they appear to be more sensitive than the conventional reference test. Many investigators repeat the test being evaluated on discordant specimens or subject them to additional tests, a strategy known as discrepant analysis. Discrepant analysis aims to identify, by an additional confirmatory assay, true-positive samples, negative by the reference method but positive by the test under evaluation. Since this procedure can improve the apparent sensitivity and specificity of the new test, selective supplemental testing favors the test being evaluated and introduces a data bias (8, 10). The size of the bias will depend on the sensitivity of the reference test and on the prevalence of disease. The lower the sensitivity of the reference test is, the higher the increase in specificity of the new test will be; the lower the prevalence of disease, the higher the increase in sensitivity (8, 15, 18). An alternative approach is to use a combination of tests to establish an expanded "gold standard" for the evaluation of a new diagnostic test (1). The introduction of an additional test to an expanded gold standard implies that it should be performed on all the specimens. This approach, however, is not common practice because it increases substantially the workload and the costs of a study (17, 19, 24). In this study the results were analyzed by applying an expanded gold standard and by discrepant analysis.

Commercial NAATs have their own specimen collection kits and transport media; unfortunately, these are incompatible, creating a difficulty for comparative studies. To overcome this inconvenience and to avoid problems of specimen collection order and bias due to interswab variation, we used single dry endocervical swabs. It has been shown that specimens transported on dry swabs have a higher positivity rate than swabs swirled for 15 s in transport medium and then discarded (14).

TABLE 7. Combined results of ELISA, PCR, and SDA for all 733 endocervical swabs tested for chlamydial infection

| Result of: | | | No. of specimens |
|---|---|---|---|
| ELISA | PCR | SDA | |
| + | + | + | 28 |
| − | + | + | 43 |
| + | − | − | 1[a] |
| − | + | − | 16[b] |
| − | − | − | 645 |

[a] Positive by LCR.
[b] Three were LCR positive, and 13 were LCR negative.

Various levels of DNA amplification inhibition with clinical specimens have been observed (20, 29, 31). Routine inclusion of an internal control as provided by PCR and SDA (not by LCR) allows the detection of amplification-inhibiting factors, validating the negative results. No inhibition was observed in our samples.

In vitro culture is still the reference method for the diagnosis of gonorrhea. The low sensitivity of *N. gonorrhoeae* culture (65%) found in our study is consistent with other publications reporting sensitivities ranging between 50 and 84% (2, 5, 6, 13). These data indicate that gonococcal infection in females as defined by culture is significantly underdiagnosed. Reasons for false-negative *N. gonorrhoeae* culture could include prior antimicrobial therapy, loss of viability of the organisms during transport, low concentrations of the organisms, or sampling error.

It has been shown in several recent studies that chlamydia culture, previously considered to be the gold standard, has a sensitivity ranging from 50 to 85% in expert laboratories (4, 12, 23). Because of logistic problems and limited resources, we did not perform *C. trachomatis* culture in this study but used classic *C. trachomatis* antigen detection by ELISA for rapid diagnosis and patient management. After confirmation of initially positive samples by a blocking assay, the specificity of this test was 100%, but the sensitivity was extremely low (38.7%). Most studies performed on endocervical specimens found sensitivities ranging between 45 and 70% (16, 25, 26, 32). In a study performed by Toye et al., 19 cases of *C. trachomatis* were detected by PCR, 8 were detected by culture, and none were detected by ELISA (29). In our study there was a wide variation of the ELISA sensitivity observed in the three participating centers, ranging from 12.8 to 63.6 to 71.4%. This variation most likely reflects a combination of differences in skills between the clinicians collecting the specimens, differences in transport and storage conditions, and variability in laboratory expertise.

In this study the performance of PCR, SDA, and LCR for the detection of *N. gonorrhoeae* and *C. trachomatis* infection was evaluated in 396 endocervical specimens, applying an expanded gold standard. No significant difference was observed between the sensitivities of PCR, SDA, and LCR, which were 95.2, 88.9, and 88.9%, respectively, for *N. gonorrhoeae* and 98, 94, and 90%, respectively, for *C. trachomatis*. For the detection of *N. gonorrhoeae* the specificities of the NAATs were more than 99% and statistically similar. For *C. trachomatis*, the spec-

TABLE 8. Performance characteristics of *C. trachomatis* ELISA, PCR, and SDA with and without supplemental testing of discordant results

| Test | Discrepant analysis[a] | Sensitivity (%) | 95% CI | Specificity (%) | 95% CI |
|---|---|---|---|---|---|
| ELISA | A | 28/71 (39.4) | 28.0–51.7 | 661/662 (99.8) | 99.2–100 |
| | B | 29/75 (38.7) | 27.6–50.6 | 658/658 (100) | 99.4–100 |
| PCR | A | 71/71 (100) | 94.9–100 | 646/662 (97.6) | 96.1–98.6 |
| | B | 74/75 (98.7) | 92.8–100 | 645/658 (98.0) | 96.6–98.9 |
| SDA | A | 71/71 (100) | 94.9–100 | 662/662 (100) | 99.4–100 |
| | B | 71/75 (94.7) | 86.9–98.5 | 658/658 (100) | 99.4–100 |

[a] A, before supplemental testing of discordant results; B, after resolution of discordant ELISA-positive and discordant PCR-positive results by LCR.

ificities of PCR and LCR were 98 and 98.6%, respectively, versus 100% for SDA; this difference was not significant.

For a second series of 337 samples PCR and SDA were performed in parallel, but LCR was done only on samples with discordant results for *N. gonorrhoeae* and *C. trachomatis*. By combining both series of samples, the performance characteristics of PCR and SDA on 733 samples were compared before and after resolution of discrepant results by LCR. For *N. gonorrhoeae* the initial sensitivities of PCR and SDA did not change significantly after resolution of discrepant results by LCR.

For *C. trachomatis* both PCR and SDA were 100% sensitive. After resolution of the PCR-SDA-discrepant results and one ELISA-positive, PCR-negative, SDA-negative result by LCR, the sensitivity of PCR decreased slightly to 98.7% and the sensitivity of SDA decreased significantly to 94.7%. After discrepant analysis the specificity of PCR increased slightly and that of SDA remained unchanged.

The disparities between the sensitivities of the three NAATs used in the present study may partly be explained by slight inoculum differences resulting from splitting of samples containing very low numbers of organisms; the use of multiple swab specimens, however, would probably have resulted in more disparities.

Although our results clearly show that sensitivity and specificity estimates for NAATs may vary slightly or significantly depending on the definition of the gold standard, it seems that all three assays are well suited to screening for genital gonorrhea and chlamydial infection in female endocervical specimens. SDA was the most accurate test in this study, being 100% specific for both *N. gonorrhoeae* and *C. trachomatis*.

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