Microorganisms mistaken as vancomycin-resistant *Staphylococcus aureus* at a cancer hospital in Lahore, Pakistan: their true identity revealed

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Staphylococcus aureus has long been a major cause of hospital-acquired infection, causing high morbidity and mortality worldwide, and the proportion of methicillin-resistant *S. aureus* (MRSA) has risen worldwide during the last two decades. This has been a growing cause for concern in Pakistan, with MRSA prevalence rates of 14–51%, depending on the study.[1–4] More worryingly is emergence of reduced susceptibility or resistance to vancomycin, at one time the only uniformly effective treatment for some staphylococcal infections.[5,6]

The Shaukat Khanum Memorial Cancer Hospital and Research Centre in Lahore, Pakistan, is a tertiary treatment hospital for cancer patients. In this setting, many of the patients will have a compromised immune system, either because of neoplastic disease or as a result of irradiation or chemotherapy. Such patients are at risk of developing severe infections and it is important that treatment is prompt and effective.[7]

During a review of microbiology reports to monitor susceptibility patterns in the hospital, four interesting cases were discovered (Table 1), all of which reported the presence of *S. aureus* and resistance to vancomycin. At the time the number of reported vancomycin-resistant strains worldwide was quite small, so this seemed unusual.

The results had been reported on the strength of a simple coagulase test (slide) and a sensitivity test carried out by inoculating the organism diluted in quarter-strength Ringers solution on Mueller Hinton agar. No controls were included and the plates (including the methicillin assay) were incubated at 37^{0}C. The following day the sensitivity patterns were read and the reports issued. Subsequently, the patients were treated successfully and follow-up samples showed no infection.

It was decided to locate the organisms and, if possible, repeat the identification and susceptibility testing to confirm the original findings. Three of the isolates were located in a refrigerator. Sweeps were taken of the best areas of growth and these were subcultured on new blood agar plates and incubated at 37^{0}C for 24–48 h to grow fresh single colonies. After overnight incubation two of the repeat cultures were shown to be pure growths of Gram-positive cocci, showing slight to moderate haemolysis on the blood agar. The third failed to grow on repeated subculture and was discarded. Unless otherwise stated, all media were obtained from Oxoid and supplied by Imran Scientific, Lahore, Pakistan.

Table 1. Cases of vancomycin-resistant *Staphylococcus aureus*.

Case	Age	Sex	Condition	Diagnosis
1	45	M	Oesophageal cancer	Wound infection
2	52	M	Myelodysplastic syndrome	Bacteraemia
3	38	M	Non-Hodgkin's lymphoma	Bacteraemia
4	18	F	Pyrexia	Bacteraemia

A slide coagulase test, using human plasma, was repeated on at least five colonies from each patient. Both were negative, although one did show some slight auto-agglutination. The organisms were also inoculated on DNase agar and a tube coagulase test performed. As a further means of identification the isolates were inoculated in the bioMérieux API-Staph system. Susceptibility was determined by agar diffusion using the disc method standardised by the National Committee for Clinical Laboratory Standards (NCCLS).[8,9]

A methicillin test was carried out by preparing a suspension of the organism in quarter-strength Ringers solution (0.5 MacFarland) and inoculating it on a quarter NaCl/nutrient agar plate with a swab. A 10-μg methicillin disc was applied and the plate incubated at 30^{0}C.[10] Appropriate control strains were included (ATCC 29213 and ATCC 25923). Eventually, the two isolates were identified as *S. lugdunensis* and *S. warneri*, and both were sensitive to vancomycin (Table 2).

In recent years, coagulase-negative staphylococci (CoNS) have emerged as important nosocomial pathogens, especially in patients whose immune systems have been compromised. This increasing importance may be due to the greater use of transient or permanent medical devices (e.g., catheters or prosthetic devices).[11] In the Lahore hospital, CoNS was the most frequently reported isolate from cases of bacteraemia, although many were discounted as insignificant as they did not fulfil the standard criteria, but a number of unusual organisms began to appear.[12]

S. lugdunensis and *S. warneri* are distributed widely over the body, although generally in small populations. However, these organisms have been implicated in serious infections such as native valve endocarditis (NVE). *S. lugdunensis* has also been found in cases of skin and soft tissue infection and in repeated abscess formation.[13,14] *S. warneri* is considered a significant nosocomial pathogen involving neurosurgical ventricular shunts, prosthetic heart valves and central venous catheters.[15,16]

In the cases examined here, all patients had been treated successfully and no further action was taken. Unfortunately, the laboratory could only repeat the identification and sensitivity testing of two of the four cases, but it did result in an alteration to the testing regime to prevent further errors.

Following introduction of the Staphylase test (Oxoid) to the laboratory, use of human plasma for the coagulase test has been discontinued, while DNase agar has been retained as a secondary method. API-Staph is reserved for patients in whom CoNS isolates are considered significant and when

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Table 2. Differentiation of isolates.

Test	*S. lugdunensis*	*S. warneri*
Site of infection	Wound	Bacteraemia
Catalase	Pos	Pos
Coagulase (slide)	Neg*	Neg
Coagulase (tube)	Neg	Neg
DNase	Neg	Neg
API profile	2716150	6230113
Penicillin	R	R
Erythromycin	R	R
Methicillin	S	S
Tetracycline	S	R
Vancomycin	S	S

*some auto-agglutination

full identification would aid treatment, or when the Staphylase test and DNase agar results are discordant. Susceptibility testing now follows NCCLS guidelines and hopefully misreporting of sensitivity patterns is now avoided.

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Antibiotic resistance and identification of uncommon Gram-negative bacteria isolated from sputum of adult patients with cystic fibrosis

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The organisms *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex are well established and recognised as major causes of lung infection in cystic fibrosis (CF) patients.[1] They are important in CF lung pathology as they initiate a vicious cycle of infection and inflammation, leading to persistent lung damage, which is mediated via the host's own immune system. The clinical management of these organisms is highly problematic in terms of extensive antibiotic multidrug resistance and infection control. Descriptions of other unusual Gram-negative bacterial species colonising the lungs of CF patients are limited and little is known about the negative contributions such unusual organisms make to the pathogenesis of the disease, including the burden of antibiotic resistance.

The Northern Ireland Regional Adult Cystic Fibrosis Unit has recently identified several bacterial species as a result of a full biochemical speciation protocol. These species also show considerable antibiotic resistance and clinical persistence/recurrence, and thus this study aims to perform an enhanced investigation of their antibiotic resistance profiles, as well as the ease with which they can be identified.

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