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Original article

Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections

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Abstract

Isolates from urine samples obtained during 1999 were indentified and their susceptibility to antimicrobial agents studied along with any production of extended-spectrum β-lactamases (ESBL) by *Escherichia coli* and *Klebsiella pneumoniae*. A total of 13 774 samples were analysed using an automatic system for the detection of bacterial ATP (Coral, USA). Of these samples, 49% were reported to be positive and uncontaminated; bacteria most frequently isolated were *E. coli* (47%), *Proteus mirabilis* (7%), *Enterococcus faecalis* (6%) and *K. pneumoniae* (5%). The susceptibility studies showed 37% *E. coli* strains resistant to amoxycillin + clavulanate 33% to cotrimoxazole and 22% to ciprofloxacin. Seven strains of *E. coli* produced ESBL. Thirteen per cent of strains were resistant to cefuroxime but only (1%) to fosfomycin. Resistance to nitrofurantoin in *K. pneumoniae* was 38%. *P. mirabilis* showed 52% resistance to cotrimoxazole and 13% *Staphylococcus aureus*, were methicillin-resistant. *E. faecalis* did not show any special resistance to normal medication. Fosfomycin continued to show high activity against Gram-negative bacilli. However, enterococci, some species of *staphylococci* and yeasts were difficult to treat empirically. ESBL were detected in the isolates of *E. coli* and there were some methicillin-resistant strains of *S. aureus*.

Keywords: Urinary tract infection; Aetiology; Antimicrobial agents

1. Introduction

Respiratory and urinary tract (UTI) infections, are the infections with the greatest level of morbidity in man. Although the most frequent aetiology continues to be *Escherichia coli* [1], it is likely that the pattern of susceptibility to antimicrobial agents has altered. Since multiple resistance mechanisms have been reported [2–4], it would be of great interest to know to what extent these are present in the clinical isolates of urine samples taken from patients with community-acquired UTI. The present paper studies the presence of phenotypic resistance in isolates of community urine samples from the University Hospital San Cecilio in 1999.

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2. Materials and methods

Our hospital is a tertiary referral institution covering the centre-west of the province of Granada (Andalusia, Spain) which has a population of 270 009 inhabitants and two Primary Health Care districts. During 1999, a total of 13 774 non-hospital urine samples were analysed using an automatic system detecting bacterial ATP (Coral, USA). Those samples with values of less than two arbitrary units were considered to have less than 10^4 CFU/ml (values of more than two arbitrary units were considered to have more than 10 000 CFU/ml and two arbitrary units are equal to 10 000 CFU/ml; cut-off for positive urine was 10 000 CFU/ml) and, barring exceptions, a negative report was issued to this effect on the same day the samples were received at the laboratory. The samples with values ≥ 2 were centrifuged at $3000 \times g$ for 10 min and examined at a 400

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× magnification (cut-off for significant leukocyturia: 4–5 leukocytes/field). It was then inoculated on to Columbia sheep blood agar and a MacConkey agar plates and incubated for 24–48 h at 37 °C.

The urine was reported to be contaminated and a further sample requested whenever more than one species of bacteria grew in the absence of leukocyturia in non-immunodepressed patients, pregnant women, elderly subjects, patients with a urethral catheter or in children under the age of 3 years. In order to be classified as contaminated in these cases, it was necessary to have more than two species of bacteria present. The presence of *Staphylococcus aureus*, *Corynebacterium urealyticum* and *Candida* spp. was assessed in all cases.

The identification of the bacteria isolated and their susceptibility to antimicrobial agents was effected using the automatic system ASM ViteK (BioMerieux, Madrid) [5]. All controls required by the manufacturer were carried out. Breakpoints from NCCLS were used for interpretation of results [6].

The following antimicrobial concentrations were assayed for Enterobacteriaceae (in mg/l): amikacin (2, 8, 32), gentamicin (0.5, 2, 8), trimethoprim–sulphamethoxazole (2/38, 8/152), imipenem (4, 8), amoxycillin with clavulanate (4/2, 8/4, 16/8), ampicillin (0.5, 4, 32), piperacillin (8, 32, 64), cefepime (4, 8, 16), cefotaxime (6, 24), cefoxitin (2, 16, 128), cefuroxime (4, 16, 64), cefazolin (4, 16), ciprofloxacin (1, 4), nalidixic acid (16), fosfomycin (4, 8, 32) and nitrofurantoin (32). The presence of extended-spectrum beta-lactamase (ESBL) was determined by studying the synergy between ceftazidime or cefotaxime and clavulanate.

For non-fermenting Gram-negative bacilli, the following concentrations of antimicrobials were used: amikacin (2, 8, 32), gentamicin (0.5, 2, 8), tobramycin (0.5, 2, 8), imipenem (4, 8), meropenem (2, 4, 8), ampicillin and sulbactam (4/2, 8/4, 32/16), piperacillin (8, 32, 64), piperacillin and tazobactam (4/4, 16/4, 64/4), ticarcillin (32, 64, 128), aztreonam (4, 8, 32), cefepime (4, 8, 16), cefotaxime (6, 24), ceftazidime (4, 8, 64), ciprofloxacin (1, 4), fosfomycin (4, 8, 32), trimethoprim–sulphamethoxazole (2/38, 8/152).

For staphylococci the antibiotics were: amikacin (16, 32), gentamicin (0.5, 2, 8), amoxycillin and clavulanate (4/2, 8/4, 16/8), oxacillin (2, 4), penicillin G (0.03, 0.25, 1, 4, 16), rifampicin (1, 4), teicoplanin (2, 4, 8), vancomycin (0.5, 4, 6, 16), trimethoprim–sulphamethoxazole (0.5/9.5, 4/76, 16/304), ciprofloxacin (0.5, 1, 2), fosfomycin (8, 16) and nitrofurantoin (64). Resistance to methicillin (MRSA) used 1 μg oxacillin disks on Mueller–Hinton agar with 2% NaCl at 30 °C.

Antibiotics used for enterococci were: ampicillin (0.25, 1, 4, 16), penicillin (0.03, 0.25, 1, 4, 16), imipenem (8, 16, 32), gentamicin (500), streptomycin (2000), teicoplanin (2, 4, 8) and vancomycin (0.5, 4, 6, 16). The detection of beta-lactamase was carried out by the nitrocephin test (BBL, England). *E. coli* (ATCC # 25922 and # 35218), *Pseudomonas aeruginosa* (ATCC # 27853), *S. aureus* (ATCC # 29213) and *Enterococcus faecalis* (ATCC # 29212 and # 51299) were used for quality control purposes.

The fungi isolated were identified using an auxonograma system (Auxacolor, Sanofi-Pasteur, France) [7] and their susceptibility was measured on solid medium using the following disks: 5-fluorocytosine (10 μg), fluconazole (15 μg), itraconazole (10 μg) and amphotericin B (10 μg) (Rosco, Denmark) [8].

3. Results

The microorganisms isolated from the 6714 (49%) positive and uncontaminated samples and their susceptibility to the antimicrobial agents are shown in Tables 1–3. A total of 2798 strains were isolated. About half of these were *E. coli* with *P. mirabilis*, *Enterococcus* spp. and *K. pneumoniae* occurring in relatively small numbers. Non-glucose-fermenting Gram-negative bacilli and other enterobacteria made up 24% of isolates; gram-positive cocci, 19% (*Staphylococcus* spp., 10% and *Streptococcus* spp., 8%) and yeasts, 8%.

The antimicrobial agents with the highest levels of activity against Gram-negative bacilli (Table 1 and Fig. 1) were amikacin, cefepime and imipenem all of which are restricted to hospital use. Cefuroxime, ciprofloxacin, fosfomycin, gentamicin and nitrofurantoin showed acceptable levels of activity. Seven strains of ESBL-producing *E. coli* were detected that were resistant to the cephalosporins and aztreonam.

Nitrofurantoin was active against all strains of *S. aureus* (Table 2) and fluconazole was active against the yeasts isolated (Table 3).

4. Discussion

This study shows the distribution of microbial species isolated from patients with UTI at a hospital in Spain and their susceptibility pattern to anti-microbial agents. These data excluded those UTI which had been treated empirically because of severe infection or therapeutic failure. This might bias some of the results obtained.

The frequency of *E. coli* in urine samples varies in different studies from 32% [9] to 86% [10,11], with intermediate values in other cases: 40% [12], 65% [11,13] and 68% [10]. Our results (47%) fit with these. Why the proportion of isolates of *E.coli* was lower than in many studies cannot be explained but it could be the large variation of different species. A recent study in France gave a higher figure (75%) [3].

Table 1
Percentage of Gram-negative bacilli susceptible to various antimicrobial agents

	E. coli (1580) *n* (%)	*K. pneumoniae* (159) *n* (%)	*P. mirabilis* (241) *n* (%)	Other enterobacteria (241) *n* (%)	*P.aeruginosa* (115) *n* (%)	*Pseudomonas* spp. (2) *n* (%)
Ampicillin	553 (35)	0 (0)	149 (62)	12 (5)	–	–
Amoxycillin-clavulanate	995 (63)	122 (77)	224 (93)	55 (23)	–	–
Cefepime	1533 (97)	159 (100)	241 (100)	229 (95)	87 (76)	2 (100)
Cefotaxime	1517 (96)	159 (100)	231 (96)	152 (63)	6 (5)	0 (0)
Cefuroxime	1375 (87)	129 (81)	234 (97)	96 (40)	–	-
Cefoxitin	1517 (96)	159 (100)	231 (96)	111 (46)	–	-
Fosfomycin	1564 (99)	113 (71)	181 (75)	94 (39)	9 (8)	0 (0)
Trimethoprim	1059 (67)	153 (96)	116 (48)	234 (97)	9 (8)	0 (0)
–sulphamethoxazole						
Nalidixic acid	1106 (70)	149 (94)	171 (71)	178 (74)	–	–
Nitrofurantoin	1469 (93)	99 (62)	0 (0)	111 (46)	–	–
Piperacillin-tazobactam	664 (42)	86 (54)	145 (60)	154 (64)	101 (88)	2 (100)
Ciprofloxacin	1232 (78)	159 (100)	217 (90)	219 (91)	78 (68)	2 (100)
Amikacin	1580 (100)	159 (100)	231 (96)	241 (100)	115 (100)	2 (100)
Imipenem	1438 (91)	159 (100)	231 (96)	231 (96)	98 (85)	2 (100)
Meropenem	–	–	–	–	105 (91)	2 (100)
Ceftazidime	–	–	–	–	101 (88)	2 (100)
Ticarcillin	–	–	–	–	82 (71)	0 (0)
Tobramycin	–	–	–	–	112 (97)	2 (100)
Aztreonam	–	–	–	–	84 (73)	0 (0)
Gentamicin	1422 (90)	159 (100)	210 (87)	234 (97)	98 (85)	2 (100)

Table 2
Percentage of Gram-positive isolates susceptible to various antimicrobial agents

	S. aureus (43) *n* (%)	Coagulase-negative staphylococci (282) *n* (%)	*Enterococcus faecalis* (185) *n* (%)
Amoxycillin-clavulanate	32 (74)	71 (25)	–
Penicillin-G	3 (6)	6 (2)	161 (87)
Oxacillin	37 (87)	79 (28)	–
Nitrofurantoin	43 (100)	268 (95)	–
Trimethoprim–sulphamethoxazole	42 (98)	175 (62)	–
Ciprofloxacin	33 (77)	169 (60)	–
Fosfomycin	27 (62)	164 (58)	–
Teicoplanin	43 (100)	274 (97)	178 (96)
Imipenem	–	–	159 (86)
Streptomycin 2000	–	–	141 (76)
Ampicillin	–	–	172 (93)
Gentamicin	32 (74)	113 (40)	157 (85)
Rifampicin	42 (97)	262 (93)	–
Vancomycin	43 (100)	279 (99)	178 (96)
Amikacin	37 (85)	133 (47)	–

Table 3
Percentage of yeasts isolated in urine susceptible to various antifungal agents

	C. albicans (128) *n* (%)	*C. glabrata* (71) *n* (%)	*C. tropicalis* (14) *n* (%)	*Candida* spp. (23) *n* (%)
Fluorocytosine	128 (100)	0 (0)	14 (100)	0 (0)
Fluconazole	128 (100)	71 (100)	14 (100)	23 (100)
Amphotericin B	128 (100)	71 (100)	14 (100)	23 (100)
Itraconazole	128 (100)	38 (50)	14 (100)	23 (100)

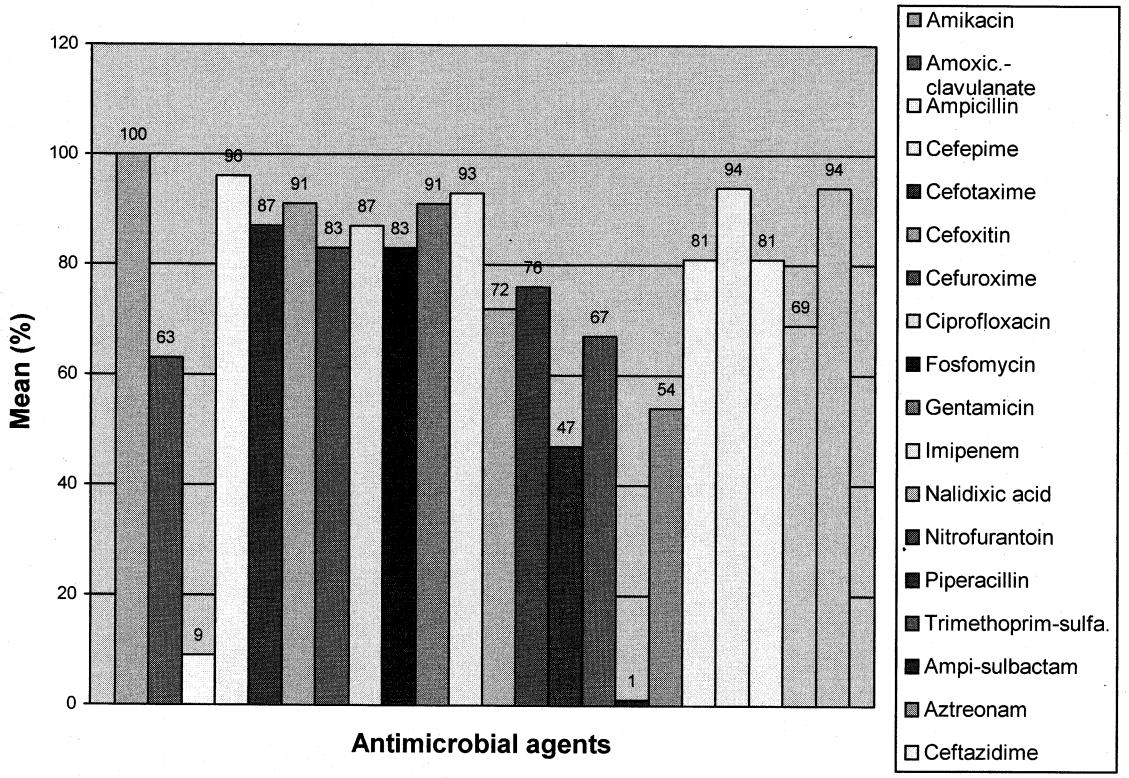

Fig. 1. Weighted average of the percentages of sensitivity of Gram-negative bacilli.

It is worth noting the considerable reduction, in our medium, in the activity of amoxycillin with clavulanate, cotrimoxazole and quinolones to *E. coli*; all drugs used in the empirical treatment of UTI. These rates confirm or contradict those of other authors. Vromen et al. [14] obtained similar values to ours for cotrimoxazole, norfloxacin and amoxycillin. Barrett et al. [13] in London, found somewhat lower levels of resistance than we did for amoxycillin (48%). In a Washington hospital [10], the resistance level for ampicillin and sulphamethoxazole was 20% and 1% for ciprofloxacin. Ahmad and Ahmad [15] found a higher resistance rate to ampicillin (86%) and other studies from USA show an increase in the resistance of *E. coli* to fluoroquinolones [16,17]. Gotlieb [18] found a resistance of 24% to cotrimoxazole. In a recent study in France, a lower level of resistance than ours was obtained for quinolones and cotrimoxazole but with similar values for amoxycillin-clavulanate [3].

P. mirabilis had a greater susceptibility to penicillins than *E. coli*, but had reduced susceptibility to cotrimoxazole (48%). Similar results were obtained by Goldstein [3]. *K. pneumoniae* was rarely found causing UTI in our series, and was highly susceptible to most antibiotics, except amoxycillin, nitrofurantoin and fosfomicina. Ciprofloxacin also showed good levels of activity, similar to Khurana et al. [19] studies from United States. Nonetheless, in view of the limited number of strains, larger studies will be required to draw definitive conclusions.

In our series, nitrofurantoin showed an acceptable level of activity against most of the microorganisms, except for *K. pneumoniae*, the *Proteus* spp. and non-fermenting Gram-negative bacilli, as found by Wolday and Erge [20] in Africa.

Seven ESBL-producing strains of *E. coli* were detected. The clinical significance of these isolates is of great importance as clinicians are 'advised against' the use of cephalosporins and aztreonam. This type of resistance has also been found in a recent study carried out in France [3].

Pseudomonas spp. were all sensitive to ceftazidime as also found by Amyes et al. [21]. The resistance rates for *E. faecalis* were similar to those found by Guirguitzova et al. [22], where over 92% were sensitive to penicillin and ampicillin, and 100% to vancomycin and teicoplanin [22]. That same study found strains with a high level of resistance to aminoglycosides (46% to streptomycin, 42% to gentamicin and 71% to amikacin). In addition, enterococci resistant to these antibiotics have been isolated in some regions of Argentina [23]. *E. faecalis* showed no special resistance to the drugs habitually used in UTI. In another study carried out by us in 1996, we found 23.5% enterococci were resistant to gentamicin [24].

Thirteen per cent of *S. aureus* strains were MRSA.

This is a high figure but less than those obtained by Ramos et al. [25], Steinberg et al. [26], Layton [27] and Goldstein [3]. These results are determined on the basis of drug concentrations in the bloodstream and the levels achieved in urine are higher, so that amoxycillin with clavulanate and the quinolones may still be effective in many cases. Cefuroxime may be used in empirical treatment but fosfomycin and nitrofurantoin were most noteworthy in terms of greater activity, economy and ease of administration. Enterococci, some species of Staphylococci and the yeasts are not, susceptible to fosfomycin. The present study also showed that multiresistant nosocomial strains (ESBL and MRSA strains) are found in the community in Spain. This may be because some patients are carriers of strains acquired during hospital stays and later disseminated in the community. However, due to the low number of staphylococcal strains studied, no definitive conclusion can be drawn in this respect. It can only be concluded that it is necessary to continue monitoring the resistance of strains isolated in community UTI.

References

[1] Sober JD, Kayer D. Urinary tract infection, Principles and Practice of Infectious Diseases, Mandell, Douglas, and Bennet's, Philadelphia 2000;1:773–805.

[2] Quintillani JR, Sahm DF, Courvalin P. Mechanisms of resistance to antimicrobial agents. In: Murray PR, Baron EJ, Pialler MA, Tenover FC, Yolken RH, editors. Manual of Clinical Microbiology, vol. 1. ASM, 1999:1505–25.

[3] Goldstein FW, The Multicentre Study Group. Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections in France.Eur J Clin Microbiol Infect Dis 2000;19:112–7.

[4] Goldstein FW, Péan Y, Gertner J, The Vigil-Roc Study Group.Resistance to ceftriaxone and other beta-lactams in bacteria isolated in the community.Antimicrob. Agents Chemother 1995;39:12 516–2 519391995

[5] Graf B, Adam T, Zill E, Gobel UB. Evaluation of the Vitek 2 system for rapid identification of yeast and yeast-like organims. J Clin Microbiol 2000;38:1782–5 May.

[6] National Committee for Clinical Laboratory Standards (2000) Performance standards for antimicrobial susceptibility testing; tenth informational supplement M100-S10. Wayne, PA: National Committee for Clinical Laboratory Standards.

[7] Paugam A, Benchetrit M, Fiacre A, Tourte-Schaefer C, Dupoy-Comet J. Comparison of four commercialized biochemical systems for clinical yeast identification by colour-producing reactions. Med Mycol 1999;37:11–7 February.

[8] Ceballos A, Gaitán LA, Orihuela F, Olea D, Ceballos L, Quindós G. Resistencia in vitro a los antifúngicos en Cándida albicans de pacientes infectados por el VIH con y sin candidosis oral. Rev Iberoamericana de Micología 1999;16:194–7 December.

[9] Okada K, Usui Y, Abe T, et al. Statistic studies on bacteria isolated from urinary tract infections. Hinyokika kiyo 1994;40:175–85 Feb.

[10] Gupta K, Scholes D, Stamm WE. Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. J Am Med Assoc 1999;281:736–8 February.

[11] Núñez Sánchez JC, Catalá Barceló MT, Navarro Obrer I, Balaguer Martínez JV. Tratamiento de las infecciones urinarias en pacientes con factores de riesgo. Med Clin 1999;24:558–9 Abr.

[12] Orret FA, Shurland SM. The changing patients of antimicrobial susceptibility of urinary pathogens in Trinidad. Singapore Med J 1998;39:256–9 June.

[13] Barrett SP, Savage MA, Rebec MP, Guyot A, Andrews N, Shrimpton SB. Antibiotic sensitivity of bacteria associated with community acquired urinary tract infection in Britain. J Antimicrob Chemother 1999;44:359–65 September.

[14] Vromen M, van der Ven AJ, Knols A, Stobberingh EE. Antimicrobial resistance patterns in urinary isolates from nursing home residents. J Antimicrob Chemoter 1999;44:113–6 July.

[15] Ahmad S, Ahmad F. Urinary tract infection at a specialist hospital in Saudi Arabia. Bangladesh Med Res Counc Bull 1995;21:95–8 December.

[16] Thomson KS, Sanders WE, Sanders CC. USA resistance patterns among UTI pathogens. J Antimicrob Chemother 1994;33:9–15.

[17] Winstanley TG, Limb DI, Eggington R, Hancock F. A ten year survey of the antimicrobial susceptibility of urinary tract isolates in the UK: Microbe Base Project. J Antimicrob Chemother 1997;40:591–4.

[18] Gottlieb PL. Comparison of enoxacin versus trimethoprim-sulfamethoxazola in the treatment of patients with complicated urinary tract infection. Clin Ther 1995;17:493–502.

[19] Khurana CM, Wojack BR. Prevalence of ciprofloxacin resistance in multiresistant gram-negative intensive care unit isolates. Infection 1994;2:s99–s104.

[20] Wolday D, Erge W. Increased incidence of resistance at Tikur Anbessa Hospital. Ethiop Med J 1997;35:127–35 April.

[21] Amyes SG, Baird DR, Crook DW, et al. A multicentre study of the in vitro activity of cefotaxime, cefuroxime, ceftazidime, ofloxacin and ciprofloxacin against blood and urinary pathogens. J Antimicrob Chemother 1994;34:639–48 November.

[22] Guirguitzova B, Chankova D, Zozikov B, Minkov N. Enterococci as uropathogens. Frequency of isolation and sensitivity to antibacterial agents. Ann Urol Paris 1998;32:15–9.

[23] Ronconi MC, Merino LA. Prevalencia de *Enterococcus faecalis* y *Enterococcus faecium* con resistencia de alto nivel a aminoglucósidos en las ciudades de Resistencia y Corrientes, República Argentina. Enfermedades Infecciosas y Microbiología Clínica, 2000 Feb;18:71–73.

[24] Gutiérrez J, Hoyos A, Piédrola G. Synergy between aminoglycosides and penicillin, teicoplanin and cefpirome in enterococci. J Antimicrob Chemother 1996;38:751–2.

[25] Ramos A, Díez A, Perz-Canosa C, Mendoza P, Montero MC. Etiología de las infecciones de vías urinarias en un hospital terciario. Enferm Infecc Microbiol Clin 2000;18:196–7.

[26] Steinberg JP, Clark CC, Hackman BO. Nosocomial and community-acquired *Staphylococcus aureus* bacteremias from 1980 to 1993: impact of intravascular devices and methicillin-resistance. Clin Infect Dis 1996;23:255–9.

[27] Layton MC, Hierholzer WJ Jr., Patterson JE. The evolving epidemiology of methicillin-resistant *Staphylococcus aureus* at a university hospital. Infect Control Hosp Epidemiol 1995;16:12–7.