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Unexpected Multidrug Resistance of Methicillin-Resistant *Staphylococcus aureus* in Urine Samples: A Single-Center Study

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Purpose: Infections of methicillin-resistant *Staphylococcus aureus* (MRSA) are becoming an increasingly concerning clinical problem. The aim of this study was to assess the development of MRSA in urine cultures in a major public university-affiliated hospital and the therapeutical and hygiene-related possibilities for reducing resistance.

Materials and Methods: This study included 243 samples from patients diagnosed with MRSA infection over a period of 6 years. An agar diffusion test measured the effects of antimicrobial agents against bacteria grown in culture. The analyses were based on the guidelines of the Clinical and Laboratory Standards Institute.

Results: A regression analysis was performed, which showed 100% resistance to the following antibiotics throughout the entire testing period: carbapenem, cephalosporin (1st–4th generation), penicillin G, aminopenicillin, β-lactamase, and isoxazolyl penicillin. However, a significant decrease in resistance was found for amikacin, gentamicin, clindamycin, levofloxacin, erythromycin, and mupirocin.

Conclusions: MRSA showed a decreasing trend of antimicrobial resistance, except against carbapenem, cephalosporin (1st–4th generation), penicillin G, aminopenicillin, β-lactamase, and isoxazolyl penicillin, for which complete resistance was observed.

Keywords: *Methicillin-resistant Staphylococcus aureus; Multidrug resistance; Urine*

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INTRODUCTION

Gram-positive bacteria have emerged as important causes of hospital- and community-acquired infections. *Staphylococcus aureus* is a leading cause of nosocomial and community-acquired infections, including bacteremia and surgical wound infections. Approximately 25% of healthy people asymptomatically carry one or more strains of *S. aureus*. Available data on the epidemiology of *S. aureus* indicate that epidemical methicillin-resistant *S. aureus* (MRSA) strains of certain phage types have increased in virulence and have spread rapidly in hospitals. Methicillin resistance was first detected in *S. aureus* in 1961 after this agent was introduced clinically. Over the past 5 decades, there has been a global epidemic of MRSA infection.

MRSA infection is usually acquired in hospitals and other health care facilities. This problem is aggravated by the tendency of MRSA for cross-infections. Heavy selection pressures toward acquiring MRSA infection are introduced by the intensive use of antibiotics, particularly cephalosporins (1st–4th generation) and carbapenem, to which organisms are resistant [1,2]. Methicillin resistance is a major risk factor for increased morbidity and mortality in *S. aureus* infections.

Bacteriuria with *S. aureus* is postulated to occur through a limited number of mechanisms that include catheterization, urologic procedures, or seeding of the genitourinary tract. Bacteremia is associated with bacteriuria in patients infected with *S. aureus*, which suggests that bacteremia is an important precursor for bacteriuria [3,4].

We hypothesized that the accurate and specific use of antibiotics could improve the resistance quota, such as in

MRSA, in the treatment of infections.

MATERIALS AND METHODS

We analyzed 5,974 pathologic urine specimens over a period of 6 years (2004 to 2009). From these, the highest infection rate was for *Escherichia coli* (3,442 cases, 57.6%), followed by *S. aureus*+coagulase-negative *S. aureus* (686 cases, 11.48%) and *S. aureus* with MRSA (243 cases, 4.06%).

The samples were plated on agar Uricult (Orion Diagnostics, Espoo, Finland) by using a dip-slide system based on 3 agar media. One side was covered with green cystine lactose electrolyte deficient-agar (CLED) medium, and the others with reddish-brown MacConkey medium and colorless *Enterococcus* medium. The system is usually used for in vitro diagnostic tests. The samples were obtained from clean, midstream voided urine or by catheterization. The agar surfaces were then completely immersed in urine and the tubes were placed in an incubator for 16 to 24 hours. To obtain a colony count (colony-forming units [CFUs]/mL), the slide was removed from the tube and the colony destiny was compared with that in the model chart provided by the manufacturer. After incubation of the inoculated slide, the presence of bacteria was detected by the formation of colonies on the agar surface. The number of colonies indicated the concentration in terms of CFU/mL. The colony count was determined by using the originally green cystine lactose electrolyte deficient-Agar (CLED) medium by matching the colony density with the model-chart item it most closely resembled. The colonies were compared in terms of number but not size. *S. aureus* colonies growing on only the CLED medium were determined to be lactose-fermenting. Such lactose-positive strains grow as yellow colonies and therefore turn the medium yellow. If the bacteria only grew on the CLED medium, Gram staining was performed. Gram-positive coccoid clusters with positive catalase reaction were identified as *Staphylococcus* spp.

If the CFU was $<10^3$, a screening on *S. aureus* and MRSA on *S. aureus* identification agar (SAID), a chromogenic medium, was required. If the CFU was $>10^3$, a bacterial suspension with McFarland no. 5 density was compounded in 0.9% saline solution by using a densitometer. The solution was added to Mueller-Hinton microbiological growth medium, and the agar diffusion test was performed. The chromogenic medium SAID was inoculated within the suspension and incubated for 16 to 20 hours in oxygen at 35°C±1°C. The results were defined per the European Committee on Antimicrobial Susceptibility Testing guidelines with cefoxitin (FOX 30 μg) as the screening agent: FOX < 22 mm indicates MRSA, whereas FOX > 22 mm indicates no MRSA infection.

All analyses were based on the guidelines of the Clinical and Laboratory Standards Institute. To assess changes in resistance over time, logistic regression was calculated for every combination of bacteria and antibiotic. The results are given as odds ratios (ORs) with 95% confidence intervals (CIs) and their respective p-values. The PASW ver. 18.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. The sampling of the specimens was in accordance with the ethical standards of the Helsinki Declaration in 1975 and the results were checked by the institutional review board.

RESULTS

During 6 years (2004–2009), we analyzed 5,974 pathologic urine specimens. Within this period, a total of 929 *S. aureus* isolates (15.55%) were recorded. The results showed 554 samples with coagulase-negative *S. aureus* (9.27%), 132 with *S. aureus* (2.2%), and 243 with MRSA (4.06%). Patients infected with MRSA were between 53 and 82 years old (mean, 77.4 years) and the male-female ratio showed a predominance of the male gender (72% vs. 28%, respectively). Of the MRSA *S. aureus* specimens, 94.3% were health-care-associated (HA-MRSA) and 5.7% were community acquired (CA-MRSA) with no signs of source (i.e., skin infection, etc.).

According to the manufacturer's protocol, the resistance to the following antibiotic substances was tested: carbapenem, cephalosporin (1st–4th generation), penicillin G, aminopenicillin, β-lactamase, isoxazolyl penicillin, gentamicin, ciprofloxacin, clindamycin, levofloxacin, erythromycin, and mupirocin.

The substances were grouped into 3 classes of antibiotics based on the results of the study. In the first group, all MRSA strains were 100% resistant to the antimicrobials, including carbapenem, cephalosporin (I–IV), isoxazolyl penicillin, penicillin G, and penicillin-B lactamase, during the 6-year period. In the second group, none of the MRSA strains were resistant to the antibiotics, including teicoplanin and vancomycin. The third group represents antibiotics against which resistance decreased during the 6-year period.

During the entire testing period, 100% resistance was observed for carbapenem, cephalosporin (1st–4th generation), penicillin G, aminopenicillin, β-lactamase, and isoxazolyl penicillin. Conversely, there were significant improvements in susceptibility among MRSA strains toward the antibiotics gentamicin, ciprofloxacin, clindamycin, levofloxacin, erythromycin, and mupirocin.

The total resistance (T) over the 6 years period was 67.08% for amikacin (OR [defining changes per year], 0.6116; $p<0.0001$), 68.31% for gentamicin T (OR, 0.6265; $p<0.0001$), 87.65% for ciprofloxacin T (OR, 0.7408; p=0.0153), 79.84% for clindamycin T (OR, 0.7914; p=0.0187), 87.24% for levofloxacin T (OR, 0.7734; p=0.0327), 79.84% for erythromycin T (OR, 0.7914; p=0.0187), and 6.69% for mupirocin T (OR, 0.6554; p=0.0226) (Table 1).

DISCUSSION

S. aureus is a common pathogen found both in the commun-

TABLE 1. Logistic regression analyses for different test antibiotics

Antimicrobial	Odds ratio (95% CI)	p-value
Amikacin	0.61 (0.51–0.74)	$<0.0001^{***}$
Gentamicin	0.63 (0.52–0.75)	$<0.0001^{***}$
Ciprofloxacin	0.74 (0.58–0.94)	0.0153^{*}
Clindamycin	0.79 (0.65–0.96)	0.0187^{*}
Macrolide	0.79 (0.65–0.96)	0.0187^{*}
Mupirocin	0.66 (0.46–0.94)	0.0226^{*}

CI, confidence interval.
Logistic regression analyses for different tested antibiotics, $^{*}p<0.05$ the potency of the used antibiotic substances shows no significant changes, $^{***}p<0.001$ represents significant decrease of resistance.

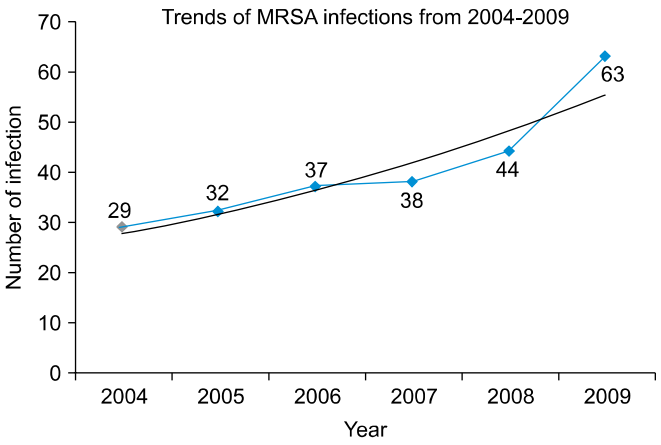

FIG. 1. Increase of methicillin-resistant *Staphylococcus aureus* (MRSA) infections during the time period from 2004 to 2009. Furthermore the exponential trend (black line) is delineated on the diagram.

ity and in hospitals. It is, however, a relatively uncommon cause of urinary tract infection in the general population [5], although isolation of *S. aureus* from urine samples is often secondary to staphylococcal bacteremia arising elsewhere. *S. aureus* is the most prominent pathogen in terms of total numbers of infections and is an important nosocomial pathogen with a high degree of nosocomial transmission [6]. This is complicated by an increasing prevalence (from 2% in 1974 to as high as 64% in 2002) of methicillin-resistant *S. aureus* among nosocomial isolates [7], which is similar to the findings of our study, which showed an increase in MRSA infection (Fig. 1).

Over the past 20 years, MRSA has emerged as an important cause of nosocomial bacteremia, and there has been a significant increase in the incidence of MRSA infections. Methicillin resistance is an additional risk factor for increased morbidity and mortality in patients with acquired *S. aureus* infections [8]. Most urinary tract infections caused by MRSA are HA-MRSA infections. Generally, these patients are asymptomatic, but in the case of a weakened general condition, a symptomatic MRSA infection can worsen the patient's status considerably and require treatment.

In relation to increasing life expectancy, patients with urinary problems, indwelling catheters, and limited mobility are in need of caution for determining sources for MRSA [9]. In this study, we found MRSA in 89.7% (n=218) of patients with catheters. The rate of infection in patients with indwelling catheters was 76.1% (n=185), and 13.6% (n=33) were patients with urinary catheters in the intensive care unit. Catheter-associated infection had a density rate of approximately 18 days. Of these infections, 10.3% (n=25) were in voided specimens.

The clinical presentation of MRSA infection is often unspectacular because the patients are asymptomatic. Often, MRSA-positive cultures are found during routine changes of indwelling catheters and no therapy is necessary. Furthermore, the symptomatic clinical differentiation between urinary MRSA and MRSA from other sites, like the bloodstream, is difficult. In this case, however, independent of the source of MRSA, treatment is implicated. The intensive care patients with symptomatic MRSA infection had septicemia in 9 cases (27.3%) because of a urinary tract infection combined with additional enterobacteria. In these patients, antibiotic combination therapy was required. In all nine cases, gentamicin was one part of the combination; the second part depended on the finding of the bacterial culture. The patients presented with fever, increased inflammatory parameters in blood, and significantly reduced general condition. In these patients, the aim is to remove any devices as soon as possible.

In patients with MRSA-induced bacteremia, a positive urine culture is typically attributed to ascending infection or to hematogenous spread. Predictors of a positive urine culture for MRSA include indwelling catheters, urinary tract obstruction, and surgery [3,10].

Contrary to HA-MRSA, CA-MRSA urinary tract infections offer clinical symptoms such as dysuria and pollakisuria. Of the 5.7% of the study population with CA-MRSA, no MRSA source was found other than the urinary sample. Possible reasons for the increase in community-acquired infections are (1) the lateral dissemination of MRSA from the hospital to the community from discharged patients diagnosed with MRSA, and (2) the discontinuation of therapy and missing follow-ups. Many strains of MRSA are frequently multi-antibiotic resistant [11]. Previous studies have suggested that MRSA infections are associated with prolonged hospitalization and increased mortality when compared with infections due to methicillin-susceptible *S. aureus*. Such comparisons may be confounded by an increased incidence of comorbid conditions among patients with MRSA infections, although the therapeutic options for patients with MRSA infections are limited. One option is selective intravenous therapy, because other common oral antimicrobials, including fluoroquinolones and third-generation cephalosporins, are ineffective against MRSA [7].

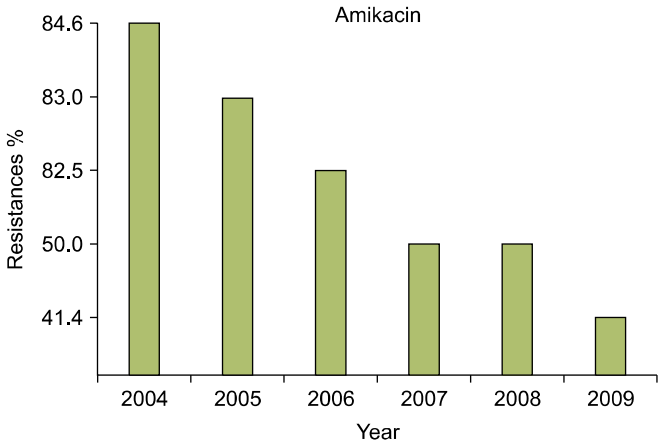

FIG. 2. Decrease in the methicillin-resistant *Staphylococcus aureus* resistance within a time period of 6 years on the basis of amikacin use.

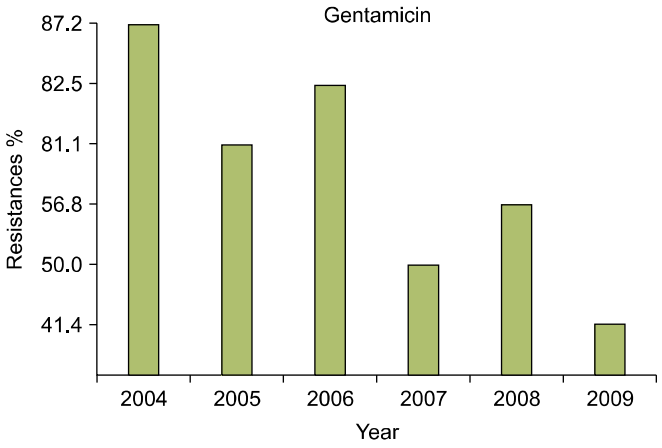

FIG. 3. Decrease in the methicillin-resistant *Staphylococcus aureus* resistance on the basis of gentamicin use within the same period as amikacin in Fig. 2.

In this study, we characterized the epidemiology of MRSA from urine cultures within the first 24 hours of hospital admission. The incidence of MRSA bacteriuria at hospital admission increased over the study period. As expected, MRSA showed complete resistance to a significant number of antimicrobial substances throughout the study. This was more common in the case of frequently used antibiotics such as aminopenicillin, cephalosporins, carbapenem, penicillin G, β-lactamase, and isoxazolyl penicillin.

Widespread use of broad-spectrum antimicrobial agents and the limited potency of some agents have exerted heavy selection pressures in hospital environments. Therefore, the re-emergence of resistant gram-positive pathogens, particularly MRSA, is of increasing concern [7].

It is difficult to eradicate MRSA in patients with indwelling catheters and stents because these bacteria form biofilms, and staphylococcal cells embedded in a biofilm or in microcolonies are conspicuously more resistant to antibiotic substances [9,12,13]. Furthermore, all involved people (i.e., medical and nursing staff, family members, and friends) can be easily contaminated and can be the link from HA- to CA-MRSA.

Therefore, is eradication necessary or does such treatment increase the resistance rate? Surprisingly, we found a significant decrease in resistance to amikacin (T, 67.08%; OR, 0.6116; $p < 0.0001$) (Fig. 2), gentamicin (T, 68.31%; OR, 0.6265; $p < 0.0001$) (Fig. 3), ciprofloxacin (T, 87.65%; OR, 0.7408; p=0.0153), and clindamycin (T, 79.84%; OR, 0.7914; p=0.0187). These findings present an indication for the specific and systemic use of antibiotics for the treatment of urinary tract infections.

As a potential source of resistance to antimicrobial agents, the noncritical use of antibiotics is supported. Even in viral respiratory infections, antibiotics were prescribed in this study. Furthermore, patient use of extant drugs for treating previous infections as well as premature termination of therapy, contrary to the recommendations, contribute to the development of resistance.

Infection control measures and screening of the nursing staff, as well as proper hand hygiene and surveillance cultures, may help to arrest the spread of MRSA in hospital settings [11,14]. An antibiotic policy may prevent MRSA and other bacteria from developing further resistance. Monitoring of susceptibility patterns of MRSA may be helpful in decreasing the prevalence of MRSA and antibiotic resistance [11,15].

Furthermore, a postdischarge collection of a self-report survey of patients who had been screened and the potential beneficial impact of MRSA screening for patients and the wider community would not reduce the infection rate but would allow an earlier and more specific therapy regimen if necessary. Furthermore, such surveys can be used to give information to involved persons about how to take appropriate hygiene measures [16].

CONCLUSIONS

The results of the present study showed that the prevalence of MRSA infections has increased in recent years. Specifically, accurate use of antibiotic substances is recommended. A decrease in selected substances over the time period was observed. These antibiotics should then be used if clinically relevant infections are present. Particularly in patients with mild symptoms of urinary tract infections and those without pathologically verified infections, no antibiotic therapy should be performed. Additionally, accurate hygiene is advised, especially in cases of contact with people in health care institutions and hospitals. Furthermore, the hospital stay of patients should be as short as possible.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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