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# ANTIBIOGRAM AND MULTIDRUG RESISTANCE PATTERNS OF STAPHYLOCOCCUS AUREUS (MDRSA) ASSOCIATED WITH POST OPERATIVE WOUND INFECTIONS IN BASRAH – IRAQ

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*SUMMARY: Fifty-two clinical samples collected from patients with postoperative wound infections in various age groups were examined for presence of multidrug-resistant bacterial pathogens – in especially – Staphylococcus aureus. The majority of samples were for age between 31 and 40 yrs (32.69%) followed by other age groups, while the lowest samples were taken from age group 1 to 10 yrs (5.76%) P<0.05. A total of 131 isolates were identified and the main causative agent was S. aureus (24 isolates 18.32%), while other bacterial types isolated from postoperative wounds were as follow: Escherichia coli 16 (12.21%), Klebsiella spp 11(8.39%), Enterobacter cloaeca 8 (6.1%), Proteus spp 7(5.34%), Pseudomonas aeruginosa 20 (15.26%), Staphylococcusepidermidis 11 (8.39%), Staphylococcus saprophyticus 8 (6.1%), Staphylococcus xylosus 5 (3.81%), Viridance streptococci 10 (7.63%), Streptococcus pyogenes 7 (5.34%), and Enterococcus faecalis 4 (3.05%) P<0.01. Sixteen antibiotics were used to test the resistance of S. aureus. Penicillin G gave 100% resistance ratio for all of 24 tested isolates, while the highly affected antibiotic was cefotaxime that gave the lowest resistance percentage (16.66%). Other antibiotics had ranges of resistance between these limits P<0.01. S. aureus developed a resistance mode for at least eight antibiotics. The biggest percentage of resistance was for the resistance of four antibiotics (21.18%) P<0.01.*

*Key words: Staphylococcus aureus MDRSA, postoperative wound infections.*

## INTRODUCTION

Infectious diseases still represent an important cause of morbidity and mortality among humans, especially in developing countries. Even though pharmaceutical companies have produced a number of new antibacterial drugs in the last years, resistance to these drugs by bacteria has increased and has now become

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a global concern. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents (1). *Staphylococcus aureus* is recognized as one of the major causes of infections in humans occurring in both the community and the hospital. *S. aureus* is a gram-positive, catalase-positive, coagulase-positive, nonmotile coccus bacterium that causes a variety of human infections in all age groups (2). It is the major causative agent in surgical wound infections and epidermal skin diseases in newborn infants (3). An *S. aureus* infection may also be superimposed on superficial dermatological diseases such as eczema, pediculosis, and mycosis (4). They live as commensals in anterior naves of over half the population of humans (5). The cocci are spread from these sites into the environment by hands, kerchief, clothing, and dust. *S. aureus* is an opportunistic pathogen in the sense that it causes an infection most commonly in tissues and sites with lowered host resistance such as in individuals with diabetes, old malnourished persons, and other chronic cases (6). *S. aureus* causes folliculitis, boil, furnculosis, scalded skin syndrome, conjunctivitis, paronychia, mastitis, and toxic shock syndrome for menstruating women who use tampons. Staphylococcal pneumonia can occur if a staphylococcal infection spreads to the lungs (7). Hospital-acquired staphylococcal infections are common in newborn babies, surgical patients, and hospital staff. Patients develop sepsis in operation wounds, which takes place in theaters during operation, and other postoperations in wards (8).

*S. aureus* is one of the most important etiological agents of many hospital-acquired infections as well as community-acquired infections and poses a constant therapeutic problem to clinicians (9). In recent years, a strong correlation between isolation of *S. aureus* and occurrence of nosocomial infections became a constant problem to hospitals and clinical centers. Methicillin and its derivatives became the drugs of choice for the treatment of infections caused by S. aureus. The appearance of multidrug-resistant *S. aureus* (MDRSA) was followed by various patterns of resistance to antibiotics (2,10). Each year some 500,000 patients in American hospitals contract a staphylococcal infection (11). The role of antibiotics is, however, more controversial when the skin is only colonized and not clinically infected (4,12). The infection rate from *S. aureus* is high. Urgent control measures should be taken to combat the renowned etiology of both nosocomial and community-acquired infection. In recent years, many isolates of *S. aureus* have evolved resistance to both synthetic and traditional antimicrobial chemotherapy, and their prevalence outside the hospital is of potential epidemiological threat (6,13). Obviously, beneficial retrospective studies on multidrug resistance must put the available conventional antibiotics in the area into consideration. Antibiotic resistance pattern of *S. aureus* (coagulase-positive). Antibiograms of MDRSA from hospitalized patients from 17 institutions in 8 countries in Asia Pacific and South Africa have shown that the proportion of MDRSA in the these regions was higher (ranging from 5.0% in the Philippines to 79.5% in Hong Kong). This proportion was higher than reported in other geographic regions contributing to over the same time: Latin America (34.9%), United States (34.2%), Europe (26.3%), and Canada (5.7%) (3,5,14).

Prolonged hospitalization and antibiotic therapy especially with lactam antibiotics predispose patients to the acquisition of MDRSA (15). Hospital-acquired MDRSA are usually associated with increased expression of multiple antibiotic resistance genes, including those coding for aminoglycoside resistance (16).

The objectives of this study were to determine the main bacterial causative agents associated with postoperative wound infections and the prevalence of (MDRSA infections in patients with postoperative wound infections in hospitals of Basrah governorate with the study of antibiogram and antibiotics resistance pattern of S. aureus.

## MATERIALS AND METHODS

### Clinical Samples

The study was undertaken in Basrah teaching and general Basrah hospitals in Basrah governorate between June and

October 2011. Fifty-two (52) clinical specimens (as a swab) were taken from various aged patients with different locations of postoperative wounds under sterile conditions.

Bacteriological technique for isolation and identification

The swab samples were collected before wound dressing. They were inoculated aseptically into sterile brain-heart infusion as transport medium and were transported to the laboratory within two hours for analysis. The samples were analyzed using the standard bacteriological media like blood agar, heated blood agar, mannitol salt agar, MacConkey agar, etc. All the bacterial isolates thus obtained were characterized and identified by studying their cultural and morphological features from the results of Gram staining reaction, serological, and biochemical tests, such as catalase, coagulase, motility, oxidase, indole, citrate utilization, urease, carbohydrate oxidation/fermentation, etc., according to the standard techniques (17,18). The main causative agents (having the highest prevalence of isolation) were tested for their susceptibility to 16 different kinds of antimicrobial agents using disk diffusion method.

Antimicrobial agents and in vitro susceptibility pattern

Only the conventional antibiotics available for frequent use by patients in the area were considered for this study. Commercially prepared disks (Hi Media, India) were used in this study (ampicillin, penicillin G, amoxicillin, erythromycin, tetracycline, gentamicin, chloramphenicol, rifapicin, tobramycin, neomycin, nitrofurantoin, ciprofloxacin, cefotaxime, amoxycillin/clavulanic acid, vancomycin, and methicillin).

The disk diffusion method for the in vitro antibiotic susceptibility test and the evaluation of the antimicrobial susceptibility were carried out according to the guidelines of the NCCLS (2002) (19), in comparison with the standard table supplies by the manufacturer.

Standardization of inoculums

Four pure colonies of each isolate on a 24-h-plate culture were randomly selected and inoculated into 2 mL of a sterile peptone water broth in Bijou bottles. This was incubated at 37°C for 6 h, and the turbidity was adjusted by serial dilution in phosphate buffer saline (pH 7. 2) to match an opacity tube containing 0. 5 mL of 1% barium chloride in 1% sulfuric acid (a Mc Farlands 0.5 barium sulfate standard containing 105 cfu/mL of the inoculums). One milliliter (1 mL) of the culture dilution (bacteria suspension) was transferred into a well-dried surface of diagnostic sensitivity test (DST) agar medium and tilted to spread evenly over the entire surface of the agar plate. The excess fluid was drained off and dried in incubator for less than 15 min. Antibiotic disks were then placed on the surface of the inoculated plate, placed in a refrigerator to allow proper diffusion of the antibiotics and incubated aerobically at 37°C for 18-24 h (overnight). *S. aureus* NCTC 6751, S. epidermidis ATCC 12228, and *E. coli* NCTC 5933 were used as control organisms for the sensitivity test. The diameter of the zone of inhibition was measured in millimeter. The result of each antimicrobial agent tested was reported as susceptible or resistant when the test organism was compared with the control and manufacturer's manuals for interpretation.

Multidrug-resistant S.aureus (MDRSA)

The percentage of the *S. aureus* that showed multiple antibiotic resistance was estimated and recorded. The percentage resistance was calculated as follows:

Percentage resistance = (No. of resistant isolates/No. of isolates tested with the antibiotic) x 100

Statistical analysis

The results were statistically analyzed by using ANOVA and T-test in the SPSS (Statistical Package for the Social Sciences) software (Version 17).

The present study was carried out with approval and agreement of Ethical and Medical Committee in College of Medicine – University of Basrah.

## RESULTS

Fifty-two clinical cases were collected from patients with postoperative wounds in various sites with various percentages as follow (Table 1):

Abdomen 10 (19.23), leg 6 (11.53), arm 5 (9.61), thigh 3 (5.76), knee 4 (7.69), head 8 (15.38), neck 7 (13.75), and chest 9 (17.30). There are no significant differences between sites of postoperative wound $P \geq 0.05$.

Table 2 illustrates the numbers of samples in various age groups. The majority of samples were taken from the age group 31-40 yrs (32.69%) followed by other age groups, while the lowest samples were taken from the age group 1-10 yrs (5.76%). There were statistically significant differences between age groups and infection $P<0.05$.

Table 1: Numbers of patients according to clinical source of isolation.

| Postoperative wounds/ or samples site | Numbers of patients | % |
|---|---|---|
| Abdomen | 10* | 19.23 |
| Leg | 6 | 11.53 |
| Arm | 5 | 9.61 |
| Thigh | 3 | 5.76 |
| Knee | 4 | 7.69 |
| Head | 8 | 15.38 |
| Neck | 7 | 13.75 |
| Chest | 9 | 17.30 |
| Total | 52 | 100 |

* There are no statistical differences between sites of postoperative wounds $P \geq 0.05$.

Table 2: Numbers of samples of post operative wounds in relation to age.

| Age | Number of samples | % |
|---|---|---|
| 1-10 | 3* | 5.76 |
| 11-20 | 5 | 9.61 |
| 21-30 | 11 | 21.15 |
| 31-40 | 17 | 32.69 |
| 41-50 | 10 | 19.23 |
| Over than 50 | 6 | 11.53 |
| Total | 52 | 100 |

* There are statistically significant differences between age groups and infection $P<0.05$.

In the present study, 131 isolates were identified and confirmed by standard techniques. These isolates belonged to various genus and types as shown in Table 3. The main causative agent having the highest isolation percentage was *S. aureus* – 24 isolates (18.32%) – while other bacterial types isolated from postoperative wounds were as follows:

*E. coli* 16 (12.21%), Klebsiella spp 11 (8.39%), Enterobacter cloaeca 8 (6.1%), Proteus spp 7 (5.34%), Pseudomonas aeruginosa 20 (15.26%), Staphylococcus epidermidis 11 (8.39%), Staphlococcus saprophyticus 8 (6.1%), Staphylococcus xylosus 5 (3.81%), Viridance streptococci 10 (7.63%), Streptococcus pyogenes 7 (5.34%), and Enterococcus faecalis 4 (3.05%).

There are highly statistically significant differences between numbers of isolates among various bacterial types $P<0.01$.

In Table 4, a total of 16 antibiotics were used to test the resistance of *S. aureus* against them. Penicillin G gave 100% resistance ratio in all of 24 tested isolates, while the highly affected antibiotic was cefotaxime that gave the lowest resistance percentage (16.66%). Other antibiotics had ranges of resistance between these limits. There are highly statistically significant differences between numbers of antibiotics resistant among *S. aureus* $P<0.01$.

The most important results in this study were shown in Table 5, which clarify the multiple resistance patterns of *S. aureus* against various antibiotics. *S. aureus* developed resistance for at least eight antibiotics, which could become a big problem in treating staphylococcal infections. The biggest percentage of resistance was for the fourth antibiotic (21.18%). No resistance recorded for 10th and 11th antibiotics. Moreover, it shows that resistance against the first antibiotic has a value of 12.5%. There are highly statistically significant differences between percentages of antibiotic-resistant species $P<0.01$.

## DISCUSSION

In the present study, the prevalence of MDRSA among patients with postoperative wound infections was found significantly higher. The majority of MDRSA

Table 3: Numbers of isolates and bacterial types isolated from patient with postoperative wounds.

| Bacterial types | Number of isolates | Isolating percentages (%) |
|---|---|---|
| Escherichia coli | 16* | 12.21 |
| Klebsiella spp | 11 | 8.39 |
| Enterobacter cloaeca | 8 | 6.1 |
| Proteus spp | 7 | 5.34 |
| Pseudomonas aeruginosa | 20 | 15.26 |
| Staphylococcus aureus | 24 | 18.32 |
| Staphylococcus epidermidis | 11 | 8.39 |
| Staphylococcus saprophyticus | 8 | 6.1 |
| Staphylococcus xylosus | 5 | 3.81 |
| Viridance streptococci | 10 | 7.63 |
| Streptococcus pyogenes | 7 | 5.34 |
| Enterococcus faecalis | 4 | 3.05 |
| Total | 131 | 100 |

* There are a highly statistically significant differences beween numbers of isolates among various bacterial types $P<0.01$.

infection isolates were found resistant to most of β-lactam antibiotics to more than four of non-lactam antibiotics. These isolates had been designated MDRSA.

*S. aureus* remains the most prominent etiology of pyogenic infections. This organism was observed in 24 (18.32%) cases of postoperative wound infections.

The cross-implication of this organism in diverse clinical cases makes it of importance to the epidemiologists. Febrile noticed among the patients could just be explained as body's immunological reaction to an infection. The trend of antibiotic resistance to a large number of commonly prescribed antibiotics observed in this study confirmed the validity of an earlier observation (20). Despite the emergence of penicillin resistance in 1942, the antibiotic is still being used to treat myriads of staphylococcal infections. The 100% penicillin G resistance observed in this research area might have emerged from the hospitals and spread to the community (7). Even the improved form of penicillin,

Table 4: Antibiotic resistance patterns of *S. aureus*.

| Antibiotic resistant | Total no. of isolates tested | Number of resistants | % |
|---|---|---|---|
| Ampicillin | 24 | 20* | 83.33 |
| Tetracycline | 24 | 16 | 66.66 |
| Gentamicin | 24 | 10 | 41.66 |
| Erythromycin | 24 | 20 | 83.33 |
| Amoxicillin | 24 | 9 | 37.5 |
| Ciprofloxacin | 24 | 7 | 29.16 |
| Rifampicin | 24 | 11 | 45.83 |
| Chloramphenicol | 24 | 15 | 62.5 |
| Ccefotaxime | 24 | 4 | 16.66 |
| Pencillin G | 24 | 24 | 100 |
| Nitrofurantoin | 24 | 10 | 41.66 |
| Tobramycin | 24 | 12 | 50.0 |
| Neomycin | 24 | 15 | 62.5 |
| Amoxycillin/Clavulanic acid | 24 | 5 | 20.83 |
| Vancomycin | 24 | 8 | 24.2 |
| Methicillin | 24 | 19 | 79.16 |

*There are highly statistically significant differences between numbers of antibiotic-resistant species among *S. aureus* $P<0.01$.

ampicillin, had 83.33% resistance thus contributing to the record of sick individuals worldwide with ampicillin-resistant infections (21). Resistance of 16.66% to the third-generation cephalosporin (cefotaxime) observed in this study can be attributed to abuse of antibiotics by illegal hospitals within the study area, since parenteral drugs are not easily abused by individuals. Incidentally, 7 of the 10 isolates that produced β-lactamase were resistant to all the β-lactam antibiotics used in this study. This revealed that the resistance is purely plasmid based since β-lactamase production is plasmid based (22). This contributed a lot to the level of multiple drug resistance, as about 21.18% of the isolates showed resistance to at least four antibiotics. Consideration of the sociodemography of the patients revealed that adults above 30 yrs dominated

Table 5: Prevalence of multidrug-resistant isolates of *S. aureus*.

| Number of Antibiotics | Number of Resistant Isolates | % |
|---|---|---|
| 1 | 3 | 12.5* |
| 2 | 2 | 8.33 |
| 3 | 2 | 8.33 |
| 4 | 3 | 12.5 |
| 5 | 5 | 21.18 |
| 6 | 4 | 16.66 |
| 7 | 2 | 8.33 |
| 8 | 2 | 8.33 |
| 9 | 1 | 4.16 |
| 10 | 0 | 0 |
| 11 | 0 | 0 |
| Total | 24 | 100 |

* There are highly statistically significant differences between percentages of antibiotic-resistant species P<0.01.

(32.69%).They could shift chemotherapy easily to drugs that were stable against β-lactamase enzyme like fluoroquinolone, which, however, could not be used by children in the first category (23). Though higher MDRSA was observed in this study, the antibiotics could provide a better therapy than the β-lactam drugs used. The reason might not be far from the stability of the drug to β-lactamase production. The resistance, however, might suggest the role of mec A gene in the isolates (24). In view of the foregoing, this would be chemotherapy of choice due to the observed low resistance of 20.83% and 24.2% to amoxicillin/clavulanic acid and vancomycin, respectively. The upsurge in the antibiotic resistance noticed in this study is in agreement with an earlier report (25) in which antibiotic abuse and high prevalence of self-medication with antibiotics were identified as being responsible for the selection of antibiotic-resistant bacterial strains.

Although the sensitivity of the organism isolated to the third-generation cephalosporin (cefotaxime) was generally excellent in the present study, the high cost of this group of drugs precludes their use as a first choice in the treatment of postoperative wound infections and septicemia; the usage policy would be made applicable to the different tiers of our health care providers at the primary, secondary, and tertiary levels. This can be done concurrently with sustained enlightenment and media publicity focusing attention on the dangers of high incidence of bacterial resistance to antibacterial agents in general and the ultimate consequences.

The trends noticed in the present study is in accordance with general trends noticed in other hospital settings (25,26,27) and in Saudi Arabia (28,29). It is noticed that MDRSA detected in the present study is higher than those reported previously in Saudi Arabia (30,31). The emergence of MDRSA causes difficulties in the treatment of infections caused by S. aureus. Antibiotic-resistant strain is a problem for the infected patients who are responding poorly to treatment and also a problem to the hospital that performs control and prevention programs. The rates of MDRSA originated from nosocomial infections.

Several risk factors for the acquisition of MDRSA among postoperative wound infections had been identified: Prolonged hospitalization, severe underlying illness in patients who are exposed to MDRSA in the hospital, and prolonged exposure to antibiotics (32,33).

Other measures that may prevent the nosocomial transmission of MDRSA include improved antibiotic stewardship, staff cohorting, maintenance of appropriate staffing ratios, reduction in length of hospital stays, contact isolation, active microbiologic surveillance, and better staff education (34). Currently, the efficacy of many of these individual infection control interventions remain in doubt. Many studies reporting improvement in infection control outcomes (e.g., reduced transmission, decreasing prevalence) involve simultaneous institution of several of these measures, making it impossible to tease out the effects of any of the individual components (35,36). High prevalence of multiple drug resistance among isolates in the present study clearly indicates the excessive or inappropriate use of antibiotics in community (37,38).

In Iraq, like in many other countries, antibiotics are readily available from the pharmacy desk. Alternatively, pharmacists prescribe medications to patients just based on their external symptoms, causing the intake of wrong antibiotic and/or over- or underdosage. Moreover, in majority of cases, patients do not complete the prescribed course of antibiotics. This causes patients to be at the hospital harboring resistant strains. These strains may cause endogenous or exogenous infections in other patients.

The higher prevalence of resistance to antimicrobial agents in this environment could be due to widespread, indiscriminate use of antibiotics. The formulation and implementation of a national drug policy by Iraqi governments are fundamental to ensure rational drug use. Proper use of drugs has to be promoted by providing objective information and training (39).

The idea of vaccine against staphylococcal infections would be a welcomed development, since vaccines are not usually available for abuse. It is time to embrace the use of local plant extract with proven therapeutic and prophylactic potency (40,41).

In conclusion, the resistance rates of *S. aureus* to antimicrobial agents among hospital-acquired infections isolates were found significantly higher ($p<0.01$).

Hospital environment is a risk factor for the prevalence of MDRSA in the hospitalized patients, visitors, and hospital staff, and may spread a major risk to the community. Efforts are therefore needed to reduce the spread of MDRSA by strict application of infection control guidelines.

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