Resistance Patterns of *Staphylococcus aureus* isolates Against Various Conventionally Used Antibiotics

Motwakel Elamin¹, Omer M.E.A², Manal Abusam¹ and Aimun AE.³

¹Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Omdurman Islamic University, Sudan.
²Department of Pharmaceutical Microbiology, Faculty of Pharmacy, ElNeeelain University, Sudan.
³Department of Pharmacology, Faculty of Medicine, Al Baha university, KSA.

**ABSTRACT**

*Staphylococcus aureus* was recognized as a major cause of nosocomial infections worldwide and a variety of skin infections which require therapeutic approaches. During the last five decades, *Staphylococcus aureus* clones that resist methicillin (methicillin-resistant *Staphylococcus aureus*, MRSA) disseminated and caused medical and public health problem worldwide 250 clinical isolates collected from Omdurman, Khartoum and Soba hospitals from different sources (blood, urine, pus, diabetic sepsis swab and tip of the catheter), cultured and isolated in pure culture and subjected to microscopical examination by the Gram reaction and biochemical tests to identify them as pure *Staphylococcus aureus*, then subjected to sensitivity tests by disc diffusion method against commonly used antibiotics in order to determine the sensitivity and resistance pattern against penicillin, co-amoxiclav, oxacillin, fusidic acid, cefuroxime, vancomycin, cotrimoxazole, rifampicin, gentamicin, clindamycin, meropenem and chloramphenicol. 110 of the pure clinical isolates were methicillin-resistant *Staphylococcus aureus* (MRSA) which were mannitol non-fermenters and resistant to number of antibiotics and the prevalence of them may increase the emergence of glycopeptide (vancomycin and teicoplanin) resistant strains. The study revealed there were many antibiotics effective against methicillin-resistant *Staphylococcus aureus* (MRSA) and can be used by the clinicians as therapeutic alternatives for the treatment of infections caused by these strains.

1. Introduction

Bacteria are said to be resistant to an antibiotic if the maximal level of that antibiotic that can be tolerated by the host does not halt their growth. Some organisms are inherently resistant to an antibiotic. For example, Gram-negative organisms are inherently resistant to vancomycin. However, microbial species that are normally responsive to a particular drug may develop more, resistant strains through spontaneous mutation or acquired resistance and selection. Some of these strains may even become resistant to more than one antibiotic [1].

The determination of antimicrobial susceptibility of clinical isolates is often crucial for the optimal antimicrobial therapy of infected patients. This need is only increasing with increasing resistance and the emergence of multidrug-resistant microorganisms. Testing is required not only for therapy but also to monitor the spread of resistant organisms or resistance genes throughout the hospital and community. Standard procedures and breakpoints have been defined to predict therapeutic outcome both in time and at different geographic locations. In some cases the presence of a resistance gene is highly predictive for clinical outcome of antimicrobial therapy. For example, the presence of a β-lactamase in *Neisseria gonorrhoeae* correlates well with the outcome of penicillin treatment. However, the presence of a resistance gene does not necessarily lead to treatment failure, because the level of expression may be too low.

For example, β-lactamase production among members of the *Enterobacteriaceae* is common, but the development of resistance is dependent on the mode and level of expression [2].

Antibiogram provides qualitative results by categorizing bacteria as susceptible, intermediate or resistant. Therefore, it is a typing tool based on the resistance phenotype of the microbial strain tested, its outcomes also guide clinicians in the appropriate selection of initial empiric treatments, and antibiotics used for individual patients in particular situations. However, since the bacterial growth inhibition does not mean the bacterial death, this method cannot distinguish bactericidal and bacteriostatic effects [3].

*Staphylococcus aureus* characterized as spheres grow characteristically in aggregates which have been likened to a bunch of grapes. The organisms are non-motile and non-sporing; they can grow aerobically or anaerobically. *Staphylococcus aureus* produces a golden yellow pigment. It is a cause of skin lesions such as boils, and can affect bone tissue in the case of *staphylococcal* osteomyelitis. A common manifestation of its infection is the production of pus, i.e. the organism is pyogenic [4]. In recent years multi-drug resistance has increased among certain pathogens which include *Staphylococcus aureus*, *Enterococci* and *Mycobacterium tuberculosis*. *Staphylococcus aureus* resistant to methicillin is...
known as methicillin-resistant *Staphylococcus aureus* (MRSA).

These strains are resistant to many antibiotics and has been responsible for major epidemics worldwide, usually in hospitals where they affect patients in high-dependency units such as intensive care units, burns units and cardiothoracic units. MRSA have the ability to colonize staff and patients and to spread readily among them. Several epidemic strains are currently circulating in the United Kingdom. The glycopeptides, vancomycin or teicoplanin, are the currently recommended agents for treating patients infected with these organisms [4].

Clinically, one of the most important examples of β-lactam resistance is that found in methicillin-resistant *Staphylococcus aureus* (MRSA) strains. These are causing increasing concern in hospitals, especially because methicillin resistance is often accompanied by multiple resistances to unrelated antibiotics. Methicillin is resistant to β-lactamases and is a mainstay in the treatment of *Staphylococcus aureus* since over 90% of hospital strains produce β-lactamase. It is capable of functioning when all other penicillin binding proteins (PBP's) have been inhibited and is sufficient to catalyse all the reactions necessary for cell growth. Resistance is mediated by the mec gene, whose origin is unknown. This is an example of resistance by duplication of an antibiotic target, the new version being resistant to the antibiotic. Mupirocin is employed topically in eradicating nasal and skin carriage of *Staphylococci*, including methicillin-resistant *Staphylococcus aureus* colonization. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are rather more resistant to biocides, especially cationic ones, than are methicillin-sensitive *Staphylococcus aureus* (MSSA) strains [4].

The resistance found in strains of *Staphylococcus aureus* began to be isolated and identified, limiting the use of several drugs over the years. In 1960, methicillin raised, a year later resistant strains were found with the emergence of Methicillin-resistant *Staphylococcus aureus* (MRSA), and the options for the treatment of infections by MRSA have become limited. Glycopeptides (vancomycin and teicoplanin) remained as the last resource for treatment of MRSA until recently. In 1996 it was first reported the emergence of VISA and it has since then been a major concern among all health professionals. The VISA would soon spread around the world and this intermediate sensitivity to vancomycin might be associated with prolonged contact of the organism to this antimicrobial agent.

Although some strains are of intermediate aspect, there is no clinical response to treatment with vancomycin. Even with appropriate therapy, patients do not show signs of improvement [5].

2. Objectives
1. To assess the resistance patterns of *Staphylococcus aureus* to antibiotics used regularly in Sudan.
2. To investigate the presence of Methicillin resistant *Staphylococcus aureus* (MRSA) in Sudan, therefore helping the clinicians to find therapeutic alternatives for the treatment of this strain.

3. Materials & Methods
3.1 Materials
3.1.1. Culture media
Mannitol salt agar, Muller Hinton agar, Deoxyribonuclease agar, Nutrient agar and Nutrient broth.

3.1.2. Chemicals and Reagents
Crystal violet, Iodine solution, Safranine red 0.5% dye, Acetone, Barium Chloride, Conc.Sulphuric acid, Normal saline (0.9% NaCl), Hydrogen peroxide solution, hydrochloric acid 3.6% (v/v) and Immersion oil.

3.1.3. Chemotherapeutic agents (Discs).
Chloramphenicol 30 µg, Rifampicin 5 µg, Penicillin 10 µg, Oxacillin 5 µg, Cefuroxime 30 µg, Fusidic acid 30 µg, Gentamicin 10 µg, Co-Trimoxazole 25 µg, Vancomycin 10 µg, Co-amoxiclav 30 µg, Meropenem 10 µg and Chindamycin 2µg.

3.2. Methodology
A total of 250 clinical isolates were obtained from wound swab, urine, and tip of catheters, then were identified primarily as *Staphylococci* in Khartoum Hospital Laboratory, Omdurman Hospital Laboratory and Soba Hospital Laboratory and then were collected. All of these clinical isolates were subjected to numbers of tests (Gram stain, catalase, DNAase and tube coagulase tests) to confirm that they were *Staphylococcus aureus*.

3.2.1. Mannitol salt agar media
Employed as a selective medium for the isolation of pathogenic *Staphylococci* which ferment mannitol giving yellow zones around the colonies. First of all, each sample of bacteria collected from Khartoum Hospital Laboratory, Omdurman Hospital Laboratory or Soba Hospital Laboratory was streaked on mannitol salt agar media aseptically and then were incubated at 37°C for 24 h. Any sample which didn’t grow on mannitol salt agar was excluded.

3.2.2. Gram Stain
All the clinical isolates were examined microscopically to study the staining properties of the organisms using the Gram’s staining technique, as well as the shapes and arrangements of the cells. Gram’s staining technique is a differential technique widely used for the routine identification and classification of bacteria [6].

A smear of each sample was prepared and fixed, then crystal violet was flooded over the smear and lifted for 30 seconds, then drained off and the smear was washed with water, then iodine solution was flooded and lifted for 1 minute, then drained off and the smear was washed with water. The smear was decolourized with ethyl alcohol for only 2-3 seconds followed by washing with water.

After that counter staining was done using safranine 0.5% for 2 minute, then drained off and the smear was washed with water. After the drying of the smear, immersion oil was applied and therefore microscopic examination was taken place.

3.2.3. Catalase Test
3%(v/v) hydrogen peroxide solution was prepared and placed 2ml volumes in small test tubes ,using clean glass rod a small portion of the culture (1 to 2 colonies) was transferred into these test tubes containing 3%(v/v) hydrogen peroxide solution and were examined for evolution of bubbles.

3.2.4. DNase(Deoxy ribo nuclease ) Test
Each DNase agar plate was divided into 4 sections by drawing lines on its bottom; each line was numbered to denote each sample to be applied to them. Each sample was spot-inoculated onto its specific numbered line on the agar, then the plates were incubated at 37°C for 24 h. After incubation, each plate was flooded with 1mol/Litre (3.6%) hydrochloric acid to precipitate unhydrolysed DNA. After standing for few minutes, each plate was examined against a dark background for the opacity or cloudiness in the agar.

3.2.5. Tube Coagulase test
It is based on the presence of the enzyme coagulase in the cell of certain bacteria; the coagulase enzyme has ability to coagulate human plasma. A 1-in-6 dilution of the sterile human plasma in saline (0.85%NaCl) was prepared and
placed 1 ml volumes of the diluted plasma in small test tubes. A colony of each sample was emulsified in a tube of the diluted plasma aseptically. Then the tubes were incubated at 37°C and were examined after 1, 2 and 4 hours for clot formation by tilting the tube through 90°.

3.2.6. Sensitivity Testing

Numerous methods have been described for testing the drug sensitivity of bacteria. The antibiotic sensitivity tests may be performed either by dilution techniques or by diffusion methods. The tube dilution method needs more equipment and personnel, and so it is not used in the routine work in most of laboratories. The diffusion techniques are widely employed because they are simple; give sharp end points, and more rapid results.

For sensitivity testing using diffusion technique the Bauer-Kirby method for rapidly growing aerobic organisms was used [6].

3.2.6. a. Plates preparation

Muller Hinton agar powder was weighed using sensitive balance and was put on clean beaker to which purified distilled water was added and mixed thoroughly, then was autoclaved at 121°C for 15 min. s, after that was poured in sterile petri dishes and was allowed to solidify.

3.2.6. b. Preparation of the inoculum

A direct colony suspension was prepared from 24 h. old non-selective media agar, the turbidity was adjusted to that of standard 0.5 McFarland, which was prepared by mixing 0.5 ml of 1.175% Barium chloride and 99.5 ml of 0.36 N sulphuric acids (the count is 10⁷).

3.2.6. c. Inoculation of the test organism in the culture medium

There are different techniques for inoculation of the test organism in the culture medium. All of these techniques were tried and compared so as to choose the most appropriate one for further experimental work.

The test organism may be inoculated in the Muller Hinton agar for diffusion tests by seeding, by flooding or by streaking. Whatever method is used, it should produce a uniform growth covering the whole surface of the plates [6]. A sterile non-toxic cotton swab on a wooden applicator was dipped into the standardized inoculum, the soaked swab was rotated firmly against the upper inside wall of the tube to express excess fluid, then the entire agar surface of the plate was streaked with the swab three times, turning the plate at 60° angle between each streaking, the inoculum was allowed to dry for 5-15 minutes.

By using aseptic technique the antibiotic discs were applied at least 24 mm apart, and then the plates were incubated at 37°C. after 24 h of incubation each plate was examined by measuring the zone showing complete inhibition and the diameters of the zones were recorded to the nearest millimeter using a calibrated instrument (calibrated ruler). The diameter of inhibition zone of each antibiotic was interpreted according to zone size interpretative chart (supplied by HIMEDIA) in accordance to performance standards for antimicrobial disc susceptibility tests (CLSI).

3.2.7. Statistical analysis of the results

The antibiotics effectiveness against the samples which collected from Omdurman hospital, Soba hospital and Khartoum hospital was analyzed using SPSS statistics (Statistical Package for Social Sciences).

4. Results

4.1. Identification of the clinical isolates

250 clinical isolates were collected from Omdurman Hospital Laboratory, Khartoum Hospital Laboratory and Soba Hospital Laboratory during the period 2012-2013. The collected isolates were purified to pure Staphylococcus aureus by proper streaking on the appropriate selective media and confirmative biochemical tests were done.

4.1.1. Coagulate test

On the basis of the results of these identification tests, it was found that 250 clinical isolates, 67 were coagulate negative Staphylococci, 183 pure Staphylococcus aureus (coagulate positive) (Table 3.1.).

The purified Staphylococcus aureus (183 samples) gave positive coagulate test (tube method) by formation of clots, sometimes the plasma was converted into a stiff gel which remained in place when the tube was tilted or inverted, and they were showed typical cultural characters of Staphylococcus aureus on blood or nutrient agar when incubated at the optimal growth temperature of 37°C for 18-24 h they form colonies 1-3 mm in diameter, smooth, low convex, glistening, densely opaque, butyrous consistency and some some strains surrounded by narrow zone of haemolysis on blood agar. Older colonies became translucent and sticky. The pigmentation was buff to gold.

4.1.2. Microscopical examination

All the purified samples (183 samples) were Gram positive cocci in cluster form, non-motile and non-spore forming.

4.1.3. The growth on Mannitol salt agar

Some samples didn’t grow on mannitol salt agar and they were excluded. Some strains had an ability to grow on mannitol salt agar without fermentation of the mannitol, therefore the colour of the agar wasn’t changed. The samples which gave positive coagulate test (183 samples) had ability to grow on mannitol salt agar.

4.1.4. DNase Test

When the samples cultured on DNA agar, most strains gave positive results by observing clear un-cloudy zone surrounding the colonies after flooding hydrochloric acid (3.6%).

4.1.5. Catalase Test

All the samples which gave positive coagulate test also gave positive catalase test which shown clearly by evolution of bubbles from (3%v/v) hydrogen peroxide solution.

A pure Staphylococcus aureus culture was therefore subjected to sensitivity testing against twelve antibiotics used conventionally for the treatment of Staphylococcus aureus infection and the results were obtained and analyzed accordingly.

The results of the biochemical tests of the clinical isolates obtained from different sources.

<table>
<thead>
<tr>
<th>Biochemical tests</th>
<th>No. of isolates* (Positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl red test</td>
<td>250</td>
</tr>
<tr>
<td>Growth on Mannitol salt agar</td>
<td>250</td>
</tr>
<tr>
<td>Mannitol fermentation</td>
<td>73</td>
</tr>
<tr>
<td>Catalase test</td>
<td>250</td>
</tr>
<tr>
<td>Coagulate test (Tube method)</td>
<td>183</td>
</tr>
<tr>
<td>DNase test</td>
<td>183</td>
</tr>
</tbody>
</table>

*Number of isolates which gave a positive result with each biochemical test.

The Majority of samples were collected from Soba Hospital (65.03%) followed by Khartoum Hospital (21.31%) and Omdurman Hospital (13.66%).

The total number of the isolates was 250 clinical isolates.

All methicillin resistant Staphylococcus aureus (MRSA) which were detected had ability to grow on mannitol salt agar without fermentation of the mannitol.
4.2. Sensitivity Testing

4.2.1. Sensitivity testing for Omdurman Hospitals clinical isolates

All the samples collected from Omdurman Hospital are sensitive to vancomycin, chloramphenicol, meropenem and clindamycin while complete resistance was shown to penicillin. High susceptibility with few resistance was shown to rifampicin (96% sensitive), gentamicin (88% sensitive), co-trimoxazole (89% sensitive), cefuroxime (84% sensitive), and fusidic acid (72% sensitive). High resistance was observed to oxacillin (88% resistant) and co-amoxycyclav (75% resistant).

4.2.2. Sensitivity testing for Soba Hospitals clinical isolates

The samples which collected from Soba Hospital showed high sensitivity to vancomycin (100%), chloramphenicol (95% sensitive) followed by meropenem (91.6% sensitive), clindamycin (89% sensitive), gentamicin (89.9% sensitive), rifampicin (83.2% sensitive), co-trimoxazole (81.5% sensitive) and fusidic acid (69.5% sensitive), while 84.9% of the samples were resistant to penicillin, 73.9% were resistant to co-amoxycyclav and 53.8% were resistant to oxacillin. The percentage of samples which were sensitive to cefuroxime is 64.7% (less than Omdurman Hospital).

4.2.3. Sensitivity testing for Khartoum Hospitals clinical isolates

All the samples which collected from Khartoum Hospital were sensitive to vancomycin and chloramphenicol. High percentage (94.9%) of the samples was sensitive to meropenem followed by co-trimoxazole (92.3% sensitive), gentamicin (89.7% sensitive), clindamycin (87.2% sensitive), rifampicin (84.6% sensitive), fusidic acid (81.9% sensitive) and cefuroxime (76.9% sensitive). There were samples which showed high percentage of resistance to some antibiotics like penicillin (95% resistant), oxacillin (61.5% resistant) and co-amoxycyclav (56% resistant).

Resistance percentage of the collected isolates to the antibiotics in each hospital.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Khartoum Hospital</th>
<th>Omdurman Hospital</th>
<th>Soba Hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>95%</td>
<td>91.6%</td>
<td>91.6%</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>89%</td>
<td>89%</td>
<td>89%</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>89.9%</td>
<td>89%</td>
<td>89%</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>83.2%</td>
<td>83.2%</td>
<td>83.2%</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>81.5%</td>
<td>81.5%</td>
<td>81.5%</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>88%</td>
<td>88%</td>
<td>88%</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>84%</td>
<td>84%</td>
<td>84%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>75%</td>
<td>75%</td>
<td>75%</td>
</tr>
</tbody>
</table>

Table 4.2. The mean of inhibition zone (mm) of each antibiotic against the samples which were collected from Khartoum Hospital.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Disc</th>
<th>STD</th>
<th>Inhibition Zone</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefuroxime</td>
<td>30µg</td>
<td>≥18</td>
<td>15-17 ≤14</td>
<td>22.6±1.834 S</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30µg</td>
<td>≥18</td>
<td>13-17 ≤12</td>
<td>30.49±0.773 S</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2µg</td>
<td>≥21</td>
<td>15-20 ≤14</td>
<td>26.79±1.517 S</td>
</tr>
<tr>
<td>Co-amoxycyclav</td>
<td>30µg</td>
<td>≥19</td>
<td>-</td>
<td>18.68±4.301 R</td>
</tr>
<tr>
<td>Co-Trimoxazole</td>
<td>25µg</td>
<td>≥16</td>
<td>11-15 ≤10</td>
<td>26.64±1.63 S</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>30µg</td>
<td>≥26</td>
<td>-</td>
<td>28.74±1.63 S</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10µg</td>
<td>≥15</td>
<td>14-15 ≤13</td>
<td>22.44±1.224 S</td>
</tr>
<tr>
<td>Meropenem</td>
<td>10µg</td>
<td>≥16</td>
<td>14-15 ≤13</td>
<td>30.64±1.363 S</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>5µg</td>
<td>≥26</td>
<td>-</td>
<td>18.15±1.813 R</td>
</tr>
<tr>
<td>Penicillin</td>
<td>10µg</td>
<td>≥29</td>
<td>-</td>
<td>12.9±1.349 R</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>5µg</td>
<td>≥20</td>
<td>17-19 ≤16</td>
<td>26.28±1.654 S</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>10µg</td>
<td>≥17</td>
<td>15-16 ≤14</td>
<td>18.54±0.864 S</td>
</tr>
</tbody>
</table>

S; Sensitive, I; Intermediate, R; Resistant .STD; Standard range, S.E.M.; Standard Error of Mean.

Table 4.3. The mean of inhibition zone (mm) of each antibiotic against the samples which were collected from Omdurman Hospital.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Disc</th>
<th>STD</th>
<th>Inhibition Zone</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefuroxime</td>
<td>30µg</td>
<td>≥18</td>
<td>15-17 ≤14</td>
<td>22.24±1.756 S</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>30µg</td>
<td>≥18</td>
<td>13-17 ≤12</td>
<td>30.8±0.860 S</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2µg</td>
<td>≥21</td>
<td>15-20 ≤14</td>
<td>31.16±0.579 S</td>
</tr>
<tr>
<td>Co-amoxycyclav</td>
<td>30µg</td>
<td>≥19</td>
<td>-</td>
<td>16.62±1.375 R</td>
</tr>
<tr>
<td>Co-Trimoxazole</td>
<td>25µg</td>
<td>≥16</td>
<td>11-15 ≤10</td>
<td>25.2±1.88 S</td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>30µg</td>
<td>≥26</td>
<td>-</td>
<td>26.36±2.576 S</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10µg</td>
<td>≥15</td>
<td>13-14 ≤12</td>
<td>21.32±1.799 S</td>
</tr>
<tr>
<td>Meropenem</td>
<td>10µg</td>
<td>≥16</td>
<td>14-15 ≤13</td>
<td>30.2±1.92 S</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>5µg</td>
<td>≥26</td>
<td>-</td>
<td>19.36±5.95 S</td>
</tr>
<tr>
<td>Penicillin</td>
<td>10µg</td>
<td>≥29</td>
<td>-</td>
<td>12.56±7.57 S</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>5µg</td>
<td>≥20</td>
<td>17-19 ≤16</td>
<td>29.6±8.83 S</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>10µg</td>
<td>≥17</td>
<td>15-16 ≤14</td>
<td>18.7±0.777 S</td>
</tr>
</tbody>
</table>

S; Sensitive, I; Intermediate, R; Resistant .STD; Standard range, S.E.M.; Standard Error of Mean.
4.3. Statistical analysis of the results

The antibiotic effectiveness against the samples which were collected from Omdurman Hospital, Soba Hospital and Khartoum Hospital was analyzed using SPSS statistics (Statistical Package for Social Sciences).


The smaller the P.value, the larger the significance because it tell the investigator that the hypothesis under consideration may not be adequately to explain the observation [11].

In this study the P.value is 0.001(less than 0.05) that mean the results which obtained from sensitivity testing are significant.

5. Discussion

5.1. Identification of the clinical isolates

On the basis of the results of these identification tests, it was found that of the 250 clinical isolates, 67 were coagulase negative Staphylococci, 183 pure Staphylococcus aureus.

5.2. Sensitivity Testing

Standard methods of sensitivity testing by diffusion or breakpoints on agar.

5.2.1. Disc diffusion method

There is no single internationally accepted method of disc diffusion testing .In the USA a modification of the Kirby–Bauer method is used .This method is recommended by the National Committee for Clinical Laboratory Standards and the World Health Organization (WHO) .Standardization of the technique control is variation, and interpretation is by comparison of inhibition zones with published tables of critical zone diameters [6].

5.2.2. Antibiotics Effectiveness

5.2.2.1. Penicillin and Oxacillin

Sattar et al. (2005) studied the susceptibility and resistance pattern of Staphylococcus aureus (50 isolates) against various brands of commonly used antibiotics. Out of 50 isolates 30(60%) were identified as coagulase positive Staphylococcus aureus which were tested for susceptibility pattern , these isolates were resistant to penicillin, 17 isolates (57%) to ampicillin, 19 isolates (63%) to cloxacillin [7]. Perwaiz et al. (2007) studied the antimicrobial susceptibility pattern of MRSA isolates from patients in a tertiary care hospital; their results indicated that out of 190 positive isolates of Staphylococcus aureus, 82 isolates (43%) were found to be MRSA [8].

Taj et al. (2010) studied the pattern of antibiotic resistance in the clinical isolates of Staphylococcus aureus, methicillin resistant Staphylococcus aureus (MRSA) and define the possible emergence of vancomycin resistant Staphylococcus aureus (VRSA) in Karachi. Out of the 450 Staphylococcus aureus isolates 174 isolates (38.6%) were found to be MRSA [9].

All the previously mentioned studies were similar to the results which obtained in this study in which out of 119 clinical isolates of Staphylococcus aureus collected from Soba hospital laboratory there were 110 isolates(93%) resistant to penicillin and 64 isolates (53.9%) resistant to oxacillin (MRSA), out of 39 clinical isolates of Staphylococcus aureus collected from Khartoum Hospital Laboratory there were 37 isolates (95%) resistant to penicillin and 24 isolates(61.5%) were resistant to oxacillin (MRSA) .All the clinical isolates(25) of Staphylococcus aureus collected from Omdurman Hospital Laboratory were resistant to penicillin while 22(88%) were resistant to oxacillin(MRSA).

5.2.2.2. Co-amoxiclav

Low resistance was found to amoxicillin-clavulanic acid (32.7%) among clinical isolates of Staphylococcus aureus which studied by Taj et al. (2010) [9]. while in the present study out of 119 clinical isolates of Staphylococcus aureus collected from Soba Hospital Laboratory there were 88 isolates (74%-resistant to co-amoxiclav, out of 39 clinical isolates of Staphylococcus aureus collected from Khartoum Hospital Laboratory there were 22 isolates (56%) resistant to co-amoxiclav. The clinical isolates of Staphylococcus aureus collected from Omdurman Hospital Laboratory76% of them were resistant to co-amoxiclav.

Differences in antimicrobial activity among related compounds are often of minor importance but can occasionally be of greater significance and may be a source of confusion to the non-specialist. This applies particularly to large classes of drugs, such as the penicillins and cephalosporins, where there has been an explosion in the availability of new agents in recent years.
The lack of antibiotic policy in Sudan, unorganized prescription and over the counter handling of antibiotics especially co-amoxiclav are the main causes of those high percentage of resistance of *Staphylococcus aureus* to co-amoxiclav which were reported in the three hospitals.

### 5.2.2.3. Cefuroxime

Low resistance was found to Cefuroxime (25%) among clinical isolates of *Staphylococcus aureus* in the present study and this agree with Taj et al., study in 2010 [9]. Out of 119 clinical isolates of *Staphylococcus aureus* collected from Soba Hospital Laboratory there were only 42 isolates (35.3%) resistant to Cefuroxime and out of 39 clinical isolates of *Staphylococcus aureus* collected from Khartoum Hospital Laboratory there were 9 isolates (23%) resistant to Cefuroxime. The clinical isolates of *Staphylococcus aureus* collected from Omdurman Hospital Laboratory only 16% of them were resistant to Cefuroxime.

### 5.2.2.4. Chloramphenicol

High resistance was found to chloramphenicol (93%) among clinical isolates of *Staphylococcus aureus* which studied by Taj et al., 2010 [9], not like the present study all the samples which were collected from Khartoum Hospital and Omdurman Hospital were sensitive to chloramphenicol. The isolates which were collected from Soba Hospital showed high sensitivity to chloramphenicol (95% sensitive).The drawbacks of chloramphenicol restrict its use therefore; the reported percentage of resistance is less.

### 5.2.2.5. Co-trimoxazole

High resistance was found to co-trimoxazole (95.6% resistant) among clinical isolates of *Staphylococcus aureus* which studied by Taj et al., 2010 [9]. Not like this study 92.3% of the samples which collected from Khartoum Hospital Laboratory were sensitive to co-trimoxazole as well as the samples which collected from Soba Hospital Laboratory (81.5% sensitive) and Omdurman Hospital (88% sensitive).

Although co-trimoxazole is broad spectrum antibacterial agent, low price in Sudan market and exert high efficacy for treatment of *Staphylococcus aureus* infection as well as Gram negative bacteria, it is less prescribed, seldom used nowadays, so retained its activity.

### 5.2.2.6. Fusidic acid

Shittu et al. (2006) studied the antimicrobial susceptibility patterns and characterization of clinical isolates of *Staphylococcus aureus* in KwaZulu-Natal province, South Africa and they found that all the clinical isolates were sensitive to fusidic acid [10]. This result was similar to the results obtained in this present study, in which out of 119 clinical isolates of *Staphylococcus aureus* collected from Soba Hospital Laboratory there were 83 isolates (70%) sensitive to fusidic acid. Out of 39 clinical isolates of *Staphylococcus aureus* collected from Khartoum Hospital Laboratory there were 32 isolates (82%) sensitive to fusidic acid. The clinical isolates of *Staphylococcus aureus* collected from Omdurman Hospital Laboratory 72% of them were sensitive to fusidic acid, that mean there were high efficacy of fusidic acid against *Staphylococcus aureus*.

### 5.2.2.7. Gentamicin

High resistance was found to Gentamicin (96.3%) among clinical isolates of *Staphylococcus aureus* which studied by Taj et al., (2010) not like this study there were high sensitivity shown to gentamicin from all samples collected [14].

Out of 119 clinical isolates of *Staphylococcus aureus* collected from Soba Hospital Laboratory there were 107 (89.9%) sensitive to gentamicin, out of 39 clinical isolates of *Staphylococcus aureus* collected from Khartoum Hospital Laboratory there were 35 (89.7%) sensitive to gentamicin. The clinical isolates of *Staphylococcus aureus* collected from Omdurman Hospital Laboratory 22 (88%) of them were sensitive to gentamicin, that means it had the same susceptibility percentage.

### 5.2.2.8. Clindamycin

Shittu and Lin(2006) studied the antimicrobial susceptibility patterns and characterization of clinical isolates of *Staphylococcus aureus* in KwaZulu-Natal province and they found that the high resistance rates of MRSA to gentamicin, erythromycin, clindamycin, rifampicin and trimethoprim, treatment of MRSA infections in this province with these antibacterial agents would be unreliable [10], while Sattar et al. (2005) studied the resistance pattern of antibiotics against clinical isolates of *Staphylococcus aureus* [7]. They found that clindamycin was the most effective among the antibiotics tested in the study against their clinical isolates. As well as this study all the samples which collected from Omdurman Hospital Laboratory were sensitive to clindamycin, high sensitivity to clindamycin was showed for the samples collected from Soba Hospital Laboratory and Khartoum Hospital Laboratory (89% and 87%, respectively), also Marcinak and Frank (2006) had study revealed that clindamycin therapy is often effective for treatment of community-associated methicillin-resistant *Staphylococcus aureus* in children, but inductive resistance can develop if the isolate exhibits macrolide resistance due to the erythromycin resistance mechanism(erm) [11].

Clindamycin is available as oral tablet in Sudan pharmacies with affordable price, but it is less prescribed for *Staphylococcus aureus* infections, it was prescribed for infections (mainly dental infections) caused by anaerobic bacteria.

### 5.2.2.9. Meropenem

Low resistance was found to meropenem (13%) in the study of Taj et al (2010) as well as this study all the samples which collected from Omdurman Hospital Laboratory were sensitive to meropenem, high sensitivity to meropenem was show for the samples which were collected from Soba Hospital Laboratory and Khartoum Hospital Laboratory (91.6% and 95%, respectively) [9]. In Sudan meropenem is reserved as last choice antibiotic for severe bacterial infections as it is broad spectrum antibiotic, therefore no emergence of resistance has been reported as well as it is very expensive make it not affordable for most patients.

### 5.2.2.10. Rifampicin

All the *Staphylococcus aureus* isolates which studied by Shittu et al.(2011) were susceptible to Rifampicin, the same results obtained by Tenover et al. (2004), while in this present study there were few percentages of resistance to Rifampicin as 15%, 4% and 17% for samples which were collected from Khartoum, Omdurman and Soba hospital laboratories, respectively [12,13].

Rifampicin is not available in Sudan as single antibiotic but combined with other anti-tuberculosis chemotherapeutic agents like isoniazid (INH), and considered as reserved drug for treatment of tuberculosis and leprosy and used for other infection.

### 5.2.2.11. Vancomycin

In the present study all the clinical isolates of *Staphylococcus aureus* were sensitive to Vancomycin, therefore it can be used as reserve antibiotic for treatment of bacterial infections caused by methicillin resistant *Staphylococcus aureus*, and should not be used regularly in...
order to avoid presence of glycopeptides resistant Staphylococcus aureus.

Table. 5.1: Resistance Patterns of clinical isolates of Staphylococcus aureus.

<table>
<thead>
<tr>
<th>Resistance Patterns**</th>
<th>No. of Resistant Isolates**</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>172</td>
</tr>
<tr>
<td>P Ox</td>
<td>119</td>
</tr>
<tr>
<td>P Ox AMC</td>
<td>80</td>
</tr>
<tr>
<td>P Ox AMC FC</td>
<td>25</td>
</tr>
<tr>
<td>P Ox AMC FC CXM</td>
<td>23</td>
</tr>
<tr>
<td>P Ox AMC FC CXM COT</td>
<td>16</td>
</tr>
<tr>
<td>P Ox AMC FC CXM Cot Rif</td>
<td>9</td>
</tr>
<tr>
<td>P Ox AMC FC CXM Cot Rif GEN</td>
<td>2</td>
</tr>
<tr>
<td>P Ox AMC FC CXM Cot Rif GEN CD</td>
<td>1</td>
</tr>
<tr>
<td>P Ox AMC FC CXM VA Cot Rif GEN CD</td>
<td>0</td>
</tr>
<tr>
<td>MRP</td>
<td>0</td>
</tr>
<tr>
<td>P Ox AMC FC CXM VA Cot Rif GEN CD</td>
<td>0</td>
</tr>
<tr>
<td>MRP C</td>
<td>0</td>
</tr>
<tr>
<td>P Ox AMC FC CXM VA Cot Rif GEN CD</td>
<td>0</td>
</tr>
<tr>
<td>MRP A</td>
<td>0</td>
</tr>
</tbody>
</table>

It was observed that from Table (5.1) the resistance patterns of Staphylococcus aureus was varied according to the number of antibiotics used and the number of resistant isolates to more than one antibiotic decreased. Therefore, using of more than one antibiotic for treatment of Staphylococcus aureus infections (with considering the interaction) have been effective. Also, following rotation manner in the using of particular antibiotic will decrease the incidence of resistance development

6. Conclusion

Staphylococcus aureus which collected from Khartoum, Omdurman and Soba hospital laboratories were resistant to penicillin, oxacillin and co-amoxiclav and had reduced sensitivity to cefuroxime and fusidic acid therefore should not be prescribed for the treatment of Staphylococcus aureus infection. This study revealed that cotrimoxazole, gentamicin, clindamycin, vancomycin, meropenem and Chloramphenicol are antibiotics available in Sudan and exerted high activity (with less resistance) against the clinical isolates of Staphylococcus aureus and can be used as therapeutic alternatives for treatment of methicillin resistant Staphylococcus aureus (MRSA) infections. There are high percentage of methicillin resistant Staphylococcus aureus (MRSA) which are resistant to number of antibiotics and the prevalence of it may increase the emergence of glycopeptidases resistant Staphylococcus aureus, therefore, we have to implement antibiotic policy and decrease the excessive use of antibiotics in order to avoid the prevalence of resistance to these antibiotics particularly vancomycin as it is considered as a drug of choice for methicillin resistant Staphylococcus aureus (MRSA). Methicillin resistant Staphylococcus aureus (MRSA) which detected were grew on mannitol salt agar without fermentation of the mannitol. The study revealed that there were therapeutic alternatives for the treatment of Staphylococcus aureus infections e.g. cotrimoxazole which gave high activity, available in Sudan pharmacies as oral tablets and suspension with low cost and affordable for most patients. Although co-amoxiclav is available in Sudan with different brands and had highly prescription rate for treatment of upper and lower respiratory tract infections it had less activity (with high resistance percentage) against clinical isolates of Staphylococcus aureus with high cost.

7. Recommendations

Lack of antibiotic policy and uncontrolled prescribing habits of antibiotics(particularly broad spectrum antibiotics) as over the counter medicine may increase the emergence of glycopeptidases antibiotics (vancomycin and teicoplanin) resistant Staphylococcus aureus and this aggravate an outbreak by presence of strains which are resistant to other antibiotics, that show the importance of antibiotics policy implementation. Preventing the emergence of multidrug-resistant organisms will require a comprehensive, systematic approach that integrates the health care and public health systems. There is need to encourage and facilitate adherence to recommended prevention and control guidelines, conduct active surveillance to detect the emergence of these organisms, and ensure vigorous antibiotic stewardship by health care providers. There is need for further studies and researches about methicillin resistant Staphylococcus aureus in Sudan and finding more therapeutic alternatives, and this study is the beginning of that trend.

8. Acknowledgments

At the very onset, the author surrenders himself before the almighty lord for blessing him with the best of what he could have had. Be it this thesis, all of it is HIS Grace, MERCY and BLESSING. He has made this possible, and the author thanks the almighty lord with all humility and surrender. In the days when role models are becoming rarer and rarer, the author had the privilege of being shaped by one of the stalwarts, Prof. Mohammed Elfatih Ahmed Omer. He was Guru and not a mere teacher to him. He ironed out the rough edges by taking interest even in the minutest of the details.

9. Declaration

The author declares that there is no conflict of interest in this research.

References


