Original article

Antibiogram, prevalence of methicillin-resistant and multi-drug resistant Staphylococcus spp. in different clinical samples

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A B S T R A C T

Methicillin-resistant Staphylococcus spp. (MRSS) are causing numerous forms of illness in humans ranging from mild to fatal infections. We need to investigate the resistant pattern for different clinical isolates to control the resistance phenomena. This study was designed to provide the resistance pattern of isolated Staphylococcus spp. from various clinical samples in Khartoum State and to elucidate the frequencies of Multidrug-resistant (MDR), Extensively drug-resistant (XDR) and pan-drug resistant (PDR). Two hundred and ten bacterial isolates were from different sources (catheter tip, sputum, vaginal swab, urine, tracheal aspirate, blood, pus, nasal swab, stool, throat swab, pleural fluid, and ear swab). Isolates were identified based on their morphological characters and biochemical reaction. Antibiotics susceptibility screening was performed using twenty-three antibiotics from eighteen classes against all isolated Staphylococcus spp. following the Clinical and Laboratory Standards Institute (CLSI) guideline. The result revealed that out of 63 Gram-positive isolated bacteria, 52 (82.5%) were Staphylococcus spp. with a high incidence of S. aureus 37(71.2%). Out of all Staphylococcus spp., 38 (73.1%) were Methicillin-resistant (MR). The prevalence of MDR was higher in S. aureus (89.2%) than in S. epidermidis (75%). All Staphylococcus spp. displayed resistance to ampicillin and penicillin, while all S. aureus were sensitive to daptomycin and fosfomycin. One isolate was XDR possible PDR, while no PDR was reported in all isolated bacteria. This study provided evidence for the antimicrobial-resistant (AMR) burden in Sudan and highlighted the need for a practical and functional stewardship program to reduce the unreasonable costs of antibiotics.

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1. Introduction

Staphylococcus spp. is a bacterial pathogen that quickly obtains antibiotic resistance (Kot et al., 2020, Boucher et al., 2009). Antibiotics are one of our most powerful tools for preventing life-threatening infections, and bacteria become antibiotic-resistant by the capability to defeat the drugs used to kill them, which is a critical global public health challenge of our time. Now, people live in an era when people die because of untreated infections due to the emergence and spread of antibiotic resistance (CDC, 2019). Moreover, microbial infections with antimicrobial-resistant strains make the disease worse by increasing the possibility of mortality and rising treatment expenses compared to disease by susceptible strains (Mulvey and Simor, 2009).

Furthermore, methicillin-resistant S. aureus (MRSA) is one of the significant problems globally which can cause both community and healthcare-acquired infections; it is reported that infections by MRSA affect more than 150,000 patients yearly in the healthcare setting alone in the European Union (Baldan et al., 2009, Köck et al., 2010), the emergence of resistant bacterial pathogens mainly due to extensive and prolonged use of antibiotics. It affects increasing morbidity and mortality rates (Tsige et al., 2020). The MRSA infection is multifold (Ben Zakour et al., 2008, Holmes...
et al., 2005) and is connected with worse outcomes, extended hospital stay, more treatment expenditure, and high mortality (Zahar et al., 2005, Shorr et al., 2006).

According to the WHO, nearly 80 % of S. aureus infections in Africa are resistant to methicillin, making the traditional antibiotics worthless for infection treatment. (WHO, 2014). Other studies in Ethiopia revealed that MRSA is a significant problem in public health (Kahsay et al., 2014; Godebo et al., 2013; Dilnessa and Bitem, 2016). In addition, in Sudan, one of the African countries with a border with Ethiopia, the prevention and governor policies are not well recognised to reduce MRSA. Antibiotics are routinely and incorrectly utilised, increasing the prevalence of drug resistance strain bacteria like MRSA. Therefore, exploring increased drug-resistant MRSA is essential for controlling and treating staphylococcal infections.

Accordingly, global epidemiological surveillance is critical to complete antimicrobial-resistant (AMR) response. Knowledge of local and provincial AMR is essential for medical selection formation. Nonetheless, there is a lack of investigation capabilities for AMR requests across Sudan, and current AMR records are sparse. According to our knowledge, no previous studies in the study area. Therefore, we aim to study the current prevalence of clinical isolates of Staphylococcus spp. and their resistance profile, including the determination of the MDR, XDR and PDR. In general, the findings of this study can be used as a source to develop a guide to reduce the burden of MRSA, and it provides modernised information that is useful for health care professionals responsible for patient supervision and monitoring the emergence of AMR.

2. Material and methods

2.1. Study population and sample size

This study was cross-sectional and conducted at three hospitals in Khartoum, Sudan (Omdurman, Soba and Bahri Teaching Hospitals). The study lasted six months, from October 2019 to March 2020. A total of 210 clinical bacterial isolates were recovered from the catheter tip, sputum, vaginal swab, urine, tracheal aspirate, blood, pus, nasal swab, stool, throat swab, pleural fluid, and ear swab samples. All specimens received in the hospital’s microbiology laboratory were included during the study.

2.2. Culturing of bacterial isolate

According to the sample sources, the specimens were cultured in XLD, MacConkey Agar and Blood agar and incubated at 37 °C for 24 h. The sample showed dense pure colonies were examined for Gram stain (Cheesbourgh, 2006).

2.3. Identification of Staphylococcus spp.

All Gram-positive bacteria (GPB) were tested for catalase production, and positive bacteria were identified by culture characteristics on Mannitol Salt Agar (MSA) and blood agar. Biochemical reactions, such as DNase and coagulase production tests, are also used to recognise the species.

2.4. Detection of methicillin-resistant Staphylococcus spp. (MRS) by cefoxitin disc screen test

The susceptibility profile of Staphylococcus spp. to cefoxitin (30 µg) was carried out on Mueller-Hinton agar plates using the disc diffusion technique. The tested bacterial isolate was suspended on physiological saline, and then the turbidity was compared with a 0.5 McFarland standard. After incubation at 35 °C for 24 h, interpretation of inhibition zone performed using CLSI guidelines 2017 as follows; S. aureus, resistant ≤ 21 mm and sensitive ≥ 22 mm whereas MRS were cefoxitin resistant. For quality control, S. aureus ATCC BAA 2313 was used for MRSA, and S. aureus ATCC BAA 1026 was used in each experiment. (CLSI, 2017).

2.5. Antimicrobial susceptibility profile of isolated Staphylococcus spp.

All Staphylococcus spp. isolates were investigated for antibiotic susceptibility using a disc diffusion test for common antibiotics used in the treatment of Staphylococcus spp infections, as following discs: azithromycin (15 µg), erythromycin (15 µg), ciprofloxacin (5 µg), moxifloxacin (5 µg), levofloxacin (5 µg), clindamycin (2 µg), linezolid (10 µg), rifampin (5 µg), teicoplanin (30 µg), mupirocin (5 µg), imipenem (10 µg), gentamicin (10 µg), ampicillin (10 µg), oxacillin (5 µg), penicillin (6 µg), amoxicillin-clavulanate (20 + 10 µg), fusidic acid (10 µg), trimethoprim/ sulfamethoxazole (1.25 + 23.75 µg), tetracycline (30 µg), daptomycin (30 µg), fosfomycin (50 µg), synergic (quinupristin and daltepristin, 15 µg), and vancomycin (30 µg). The diameter of the inhibition zone was interpreted as resistant, intermediate and susceptible according to CLSI guidelines 2017. S. aureus ATCC 25,923 was used for quality control (CLSI, 2017).

2.6. Detection of MDR and XDR

MDR was defined as acquired resistance to at least one antimicrobial agent from 3 or more antimicrobial classes. XDR is characterised by resistance to at least one drug in all antibiotic types except two or fewer; bacterial isolates are only sensitive to one or two categories. The PDR is resistant to all groups of antimicrobial agents (Basak et al., 2016).

2.7. Statistical analysis

Statistical package for social sciences (SPSS) software programme version 20 (SPSS Inc., Chicago, IL) was used for analysing all data. Descriptive data is displayed as a percentage. A P-value of ≤ 0.05 was considered for significant association between sources of samples and isolated bacteria.

3. Results

3.1. Identification of samples from different sources

Out of two-hundred and ten isolates, 63 (30 %) were GPB, of this, S. aureus (58.7 %), S. epidermidis (12.7 %), S. hemolyticus (4.8 %), S. cohnii (1.6 %), S. hominis (1.6 %), S. sciuri (1.6 %), S. lugdunensis (1.6 %), Enterococcus spp. (6.4 %), Streptococcus spp. (11.2 %). Most of the isolated bacteria were from nasal swabs, pus, blood culture, and urine (Table 1).

Out of 63 GPB, Staphylococcus spp. was 52 (82.5 %) isolates as follows: S. aureus 37(71.2 %), S. epidermidis 8(15.4 %), S. hemolyticius 3(5.8 %), and 1(1.9 %) for S. cohnii, S. hominis, S. sciuri and S. lugdunensis.

3.2. Detection of methicillin-resistant Staphylococcus spp. (MRSS) by cefoxitin disc screen test

Of 52 isolated Staphylococcus spp., 38 (73.1 %) were positive for the Cefoxitin disc screen. The results are demonstrated in Table 2.
3.3. Detection of MDR, XDR and PDR

MDR was predominant. Of all isolated species, only *S. cohnii* are resistant to all tested antibiotics except for mupirocin which was not tested, so it is considered XDR and possible PDR. The results are displayed in Table 2.

3.4. Antimicrobial susceptibility profile of isolated *Staphylococcus* spp.

Twenty-three different antibiotics belong to eighteen different classes used in this study, and the result was interpreted according to CLSI guidelines. All *S. aureus* were sensitive to daptomycin and fosfomycin, and only two isolates were resistant to teicoplanin and rifampin. While all *Staphylococcus* spp. were resistant to penicillin and ampicillin, the result is shown in Table 3.

4. Discussion

In this study, we found the most common isolated species is *S. aureus* 37(71.2 %), followed by *S. epidermidis* 8(15.4 %); this might be because *S. aureus* is more virulent and *S. epidermidis* is an opportunistic pathogen (Otto, 2009). MRSA prevalence was 67.6 %, and this finding is the highest one when comparing it between African and non-African countries; such as the MRSA reported in Kenya (53.4 %) (Wangai et al., 2019), Uganda (41 %) (Ojulong et al., 2010), Addis Ababa (13.2 %) (Tsige et al., 2020), Eritrea (9 %) (Naik and Teclu, 2009), Cameroon (13.16 %) (Bissong et al., 2016), Nigeria (5.8 %) (Ghebremedhin et al., 2009), Tanzania (4.3 %) (Mshana et al., 1970) and Brazil (5.6 %) (Almeida et al., 2014). This high frequency of MDR in our study could be related to a high level of antibiotic use, which could be due to accessibility or low purchase prices of drugs.

In this study, all *Staphylococcus* spp. including *S. aureus* was resistant to penicillin and ampicillin. This finding is unlike the results obtained from Tanzania (97 %) (Mshana et al., 1970), Nigeria (95.8 %) (Uwaezuoke and Arieratu, 2004), and ultimately agreed with a study done in Ethiopia (East border country) which found that all MRSA were 100 % resistant to penicillin and ampicillin (Kejela and Bacha, 2013).

Interestingly, All *S. aureus* were sensitive to daptomycin and fosfomycin, and numerous proportions of *S. aureus* isolates, includ-

### Table 1

| Gram-positive bacteria | Source of samples | Total |
|------------------------|-------------------|-------|
|                        | vaginal swab      |       |
|                        | urine             |       |
|                        | sputum            |       |
|                        | blood             |       |
|                        | tracheal aspirate |       |
|                        | pus               |       |
|                        | throat swab       |       |
|                        | nasal swab        |       |
|                        | ear swab          |       |
| *S. aureus*            | 1                 | 37    |
| % 1.6                  | 3.2               | 3.2   | 1.6  | 4.8  | 13 | 0 | 15 | 0 | 37 |
| *S. epidermidis*       | 0                 | 0     |
| % 0.0                  | 1.6               | 0.0   | 6.3  | 0.0  | 3.2 | 0.0 | 0.0 | 1.6 | 8  |
| *S. heamolyticus*      | 0                 | 0     |
| % 0.0                  | 3.2               | 0.0   | 1.6  | 0.0  | 1   | 0.0 | 0.0 | 0.0 | 1  |
| *S. cohnii*            | 0                 | 0     |
| % 0.0                  | 0.0               | 0.0   | 1.6  | 0.0  | 1.6 | 0.0 | 0.0 | 0.0 | 1  |
| *S. hominis*           | 0                 | 0     |
| % 0.0                  | 0.0               | 0.0   | 0.0  | 0.0  | 1   | 0.0 | 0.0 | 0.0 | 1  |
| *S. sciuri*            | 0                 | 0     |
| % 0.0                  | 0.0               | 0.0   | 1.6  | 0.0  | 1   | 0.0 | 0.0 | 0.0 | 1  |
| *S. lugdunensis*       | 0                 | 0     |
| % 0.0                  | 0.0               | 0.0   | 0.0  | 0.0  | 1   | 0.0 | 0.0 | 0.0 | 1  |
| Enterococcus spp.      | 0                 | 0     |
| % 0.0                  | 0.0               | 0.0   | 1.6  | 0.0  | 1   | 0.0 | 0.0 | 0.0 | 1  |
| Streptococcus spp.     | 0                 | 0     |
| % 0.0                  | 0.0               | 3.2   | 1.6  | 0.0  | 1.6 | 1.6 | 4.8 | 0.0 | 11.2|
| Total                  | 1                 | 8     |
| % 1.6                  | 12.7              | 6.3   | 14.3 | 6.3  | 27.0| 1.6 | 28.6| 1.6 | 100.0|
| P-value                | 0.001             |       |

### Table 2

| Bacteria     | Number and percentage of isolates | MDR YES | MDR NO | MRSS (Cefoxitin test) |
|--------------|----------------------------------|---------|--------|-----------------------|
| *S. aureus*  | N 37                             | 33      | 4      | 25                    |
| % 100        | 89.2                             | 10.8    | 67.6   | 32.4                  |
| *S. epidermidis* | N 8                              | 6       | 2      | 7                     |
| % 100        | 75                               | 25      | 87.5   | 12.5                  |
| *S. heamolyticus* | N 3                              | 3       | 0      | 3                     |
| % 100        | 100                              | 0       | 100    | 0                     |
| *S. cohnii*  | N 1                              | 1       | 0      | 1                     |
| % 100        | 100                              | 0       | 100    | 0                     |
| *S. hominis* | N 1                              | 1       | 0      | 1                     |
| % 100        | 100                              | 0       | 100    | 0                     |
| *S. sciuri*  | N 1                              | 1       | 0      | 1                     |
| % 100        | 100                              | 0       | 100    | 0                     |
| *S. lugdunensis* | N 1                             | 1       | 0      | 1                     |
| % 100        | 100                              | 0       | 100    | 0                     |
| P-value      | 0.001                            | 0.001   | 0.001  | 0.001                 |

Key: MDR: multidrug-resistant bacteria, MRSS: Methicillin-resistant *Staphylococcus* spp.
Unfortunately, our results showed that S. cohnii was resistant to all tested antibiotics, with an exception for mupirocin. This result is alarming because MRSA has been exposed to antibiotics (CDC, 2019). As previously reported in the literature, S. aureus develops resistance to several antibiotics by mutations, and expressing endogenous efflux pumps (Foster, 2017). MRSA is an important human pathogen with growing resistance to presently used antimicrobial remedies (Pokharel et al., 2019).

5. Conclusion

This study focused on the current scenario of antimicrobial-resistant Staphylococcus spp. and highlighted the frequencies of MDR, XDR, and PDR among clinical isolates of Staphylococcus spp. in Khartoum state Sudan and the incidence of MDR Staphylococcus spp. was high.

Also, this study showed the current resistant patterns, which are essential for the active treatment of diseases caused by MDR and MRSA. This study might evidence the serious need for monitoring and dealing with the development of MDR strain. Finally, this study recommended that antibiotic use be improved, reduce unnecessary uses, and ensure improved access to antibiotics in Sudan.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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