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Antibiotic resistance and β-lactamase production and biofilm formation by MRSA Isolated from Dakahlia Governorate, Egypt

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) causes a wide range of infections in humans. These infections include pneumonia, abscesses, osteomyelitis, endocarditis, phlebitis, mastitis, and meningitis. This study aimed to investigate MRSA prevalence among patients, outpatients, and health care workers (HCWs) at tertiary university hospitals in Mansoura, Dakahlia. The strains were collected from different sources (puss, blood, eye, urine, HCWs) and PCR was used to detect mecA. The isolates were investigated for their susceptibility to different antibiotic classes and their ability to produce β-lactamases and biofilms. Out of the 200 S. aureus strains, 103 (51.5%) were MRSA. The prevalence of MRSA among inpatients, outpatients, and HCWs was 43.5%, 46.0%, and 61.7%, respectively. MRSA isolates were tested for their susceptibility to 15 antibiotics. More than 50% of the MRSA isolates were resistant to eight antibiotics. Less than 10% of the isolates were resistant to amikacin, ciprofloxacin and lomefloxacin. Alarming resistance levels for vancomycin (23.30%) and linezolid (56.31%) were observed. MRSA strains were multidrug-resistant (MDR) and were resistant to 4-13 antibiotic classes. All MRSA strains were biofilm producers. The strong, moderate and weak biofilm producers were 23.30%, 49.51% and 27.18%, respectively. The percentages of β-lactamase production by inpatients, outpatients, and HCWs were 90%, 95.7%, and 96%, respectively. We may conclude that MRSA isolates are prevalent in outpatients, inpatients, and HCWs in university tertiary hospitals at Mansoura. All MRSA were MDR-resistant and biofilms and most of them were β-lactamase producers. Therefore, infection control procedures must be urgently implemented.

Keywords: *MRSA, mecA, HCWs, Biofilm formation, β-lactamase.*

1. Introduction

Staphylococcus aureus is a leading cause of bacterial infections. It causes illnesses ranging from mild skin infections to bacteremia, which are associated with high mortality and morbidity rates (Reichmann and Pinho, 2017).

Although, the discovery of penicillin G in the early 1940s drastically improved prognosis, soon by 1942, resistant strains were recognised. The resistance of these strains to penicillin was through the production of a penicillinase (β-lactamase) enzyme. This enzyme hydrolyses the β-lactam ring and inactivates the drug. The β-lactamase enzyme is encoded by blaZ gene, which is located on a large transposon on a plasmid (Torgersen et al., 2003).

Methicillin is a semi-synthetic β-lactam, introduced in 1959 to circumvent penicillin resistance associated with β-lactamase enzyme production. However, in 1961, Methicillin-resistant strains of S. aureus (MRSA) were first recognized. Since then, MRSA isolates have increased and become one of the most important nosocomial and community-acquired pathogenic bacteria (Al sweify et al., 2020). The gene for methicillin resistance, mecA was found on the chromosome (Lovering, et al., 2012).

mecA encodes for the penicillin-binding protein 2A (PBP2A). PBP2A is a transpeptidase that helps to synthesise the bacterial cell wall. It has a low affinity for most β-lactams (Lovering, et al., 2012). Therefore, MRSA isolates are treated by non-β-lactam classes of antibiotics. However, this is challenged by the ability of MRSA isolates to develop resistance to the newly introduced antibiotics and to become multidrug-resistant (Al-mohana et al., 2018).

The primary pathogenic mechanism that causes S. aureus infections to become chronic and irreducible is its ability to produce a biofilm (Silva et al., 2021). Proteins, extracellular DNA, amyloid fibrils and polymeric polysaccharide adhesins constitute the staphylococcal biofilm. Two global regulators, the accessory gene regulator (agr) and staphylococcal accessory regulator (sarA) have been linked to the development of

biofilms (Vu, et al., 2009). When the bacterial population in biofilms reaches a significant quorum, agr expression causes upregulation of several virulence factors to thwart the host immune response and support the seeding dispersal of S aureus. SarA induces attachment and MRSA biofilm formation elsewhere (Munteanu, et al., 2017).

In this study, we have collected MRSA isolates from inpatients, outpatients and health care workers (HCWs) of tertiary university hospitals at Mansoura City, Dakahlia. MRSA isolates were investigated for their susceptibility to antibiotics, ability to produce β-lactamase enzymes and to form a biofilm.

2. Material and methods

Specimens

Between April 2020 and February 2021, non-duplicate samples of pus, urine, blood cultures, and nasal nares swabs were tested for S. aureus. The samples were gathered from several tertiary hospital departments, outpatient clinics and HCWs at Mansoura University tertiary hospitals, Dakahlia. Patients who spent more than 72 hours in tertiary hospitals were considered hospital-acquired. S. aureus isolates collected from patients who had not recently been hospitalised. They were considered community-acquired strains and were obtained from outpatient departments. S. aureus strains of the healthcare professionals were collected from physicians, nurses, lab technicians, and housekeepers.

MRSA isolation and identification

Standard techniques were used to identify the isolates as S. aureus. The isolates were plated out in different culture medium, such as blood agar, mannitol salt agar, and nutrient agar. To confirm the morphologically suspected staphylococcal colonies, Gram-staining, catalase, and coagulase assays were carried out (El-Baghdady, et al., 2020). S. aureus isolates were maintained at -80°C in a tryptone-soy broth (TSB) containing 20% glycerol till used. Disk diffusion susceptibility test for cefoxitin (30µg) was used for the preliminary screening of S. aureus isolates for MRSA (CLSI, 2015).

Antibiotic susceptibility testing

The minimum inhibitory concentration (MIC) for different antibiotics was carried out in accordance with CLSI guide lines.

PCR detection of the genes of *mecA*

DNA was extracted from the MRSA isolates by boiling method (Sheneef, et al., 2017). The primers used for mecA detection were FW, AAA ATC GAT GGT AAA GGT TGG C and RV, AGT TCT GCA GTA CCG GAT TTG C (Wichelhaus, et al., 1999).

The thermocycler was programmed for an initial denaturation at 94°C for 4 min, followed by 30 amplification cycles. The cycles were denaturation at 94°C for 30s, annealing at 55°C for 30s and extension at 72°C for 30s. The final extension was at 72°C for 2 min.

Iodometric–overlay method (1OM) for detection of β-lactamases

Isolates were tooth-picked onto the surface of a nutrient agar plate containing 2µg/ml penicillin G (PNG). After overnight incubation at 37°C, the plates were overlaid with 1% molten agarose containing 0.2% soluble starch and 1% PNG. The plates were incubated for 15 min at room temperature. Logul's Iodine solution was poured onto the surface of agar plates to cover them uniformly. After 10 seconds, the residual iodine solution was damped out and the plates were incubated at room temperature for a few minutes until discolouration zones appeared around β-lactamase producing colonies (Abo-Kamar and Shohayeb 1998).

Detection of biofilm formation

Each strain was subcultured onto Mueller Hinton agar, and incubated overnight at 37°C. Few colonies were suspended in sterile saline and their turbidity was adjusted to 0.5 McFarland standard and the suspension was vortexed for at least 1 min. Wells of a sterile flat-bottomed 96-well polystyrene microtitre plates were filled with 180µl of TSB supplemented with 1% glucose. Twenty µl the prepared bacterial suspensions were added to each well. The negative control wells contained only broth. After incubation, for 18h, the contents of the wells were decanted and washed three times with 300 ml of sterile phosphate-buffered saline (pH 7.2). Methanol (200µl) was used to fix the adherent microorganisms. After 20min, the plated were decanted and left inverted at room temperature to the next day for air drying. The formed biofilm layer in each microtitre plate well was stained with 150 µl of gram staining crystal violet for 15 min at room temperature. Each well was aspirated with a pipette and the excess stain was rinsed off under running tap water until the washings were free of any stain. The optical density of each well was measured at 570 nm using a microtitre-plate reader (Stepanovic, et al., 2007)

Results

Out of 200 strains of *S. aureus,* 103 (51.5%) were identified as MRSA. The distribution of MRSA in different in-patients clinics, out-patient and health care workers were 43.5%, 46.0% and 61.7%, respectively (Fig: 1).

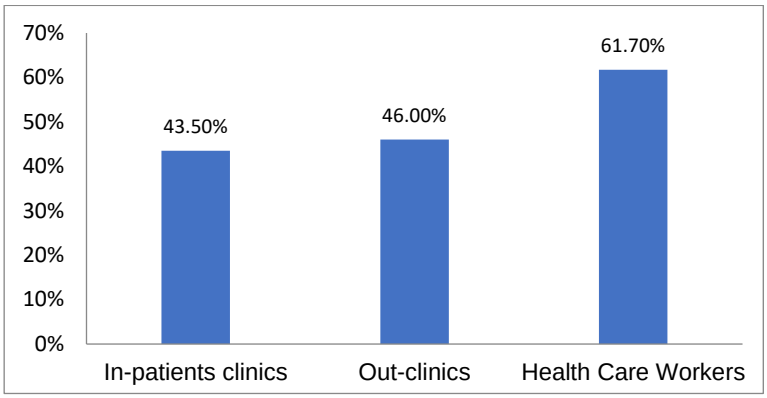

Fig 1: Distribution of MRSA isolates in inpatient, outpatient and health care workers.

Table 1 : Distribution of MRSA in different clinical isolates

Source	MRSA
Eye	14/30 (46.67%)
Puss	14/41 (34.15%)
Urine	10/28 (35.71%)
Blood	15/30 (50.00%)
Health workers	50/71 (70.42%)
Total number	103/200 (51.50%)

MRSA isolates were collected from different sources. The highest prevalence of MRSA was detected in HCWs (70.42%), followed by blood samples (50.00%) (Table 1).

MRSA isolates were tested for their susceptibility to fifteen antimicrobials belonging to nine antibiotic classes. All MRSA strains were resistant to cefoxitin antibiotic which was initially used for their detection (Table 2).

High resistance levels of the MRSA isolates were observed to gentamicin (76.69%), tetracycline (82.52%), azithromycin (94.17%), erythromycin (96.11%), and fusidic acid (96.12%), (Table 2). The resistant strains to linezolid, levofloxacin, clarithromycin, co-trimoxazole ranged between 45.63 and 56.31% (Table 2).

Low resistance levels were observed for MRSA strains to amikacin (4.85%), ciprofloxacin (6.79%) and lomefloxacin (8.73%), (Table 2). The resistance levels to vancomycin and imipenem were 22.33% and 23.30%, respectively.

All MRSA strains were multidrug resistant (MDR), (Fig. 2). The resistance patterns MRSA isolates were very diverse (data not shown). The isolates were resistant to 4 to 13 antibiotics. Most of the MRSA isolates (95.13%) were resistant to 5-11 antibiotics (Fig. 2). The per cent of resistant isolates to 4, 12 and 13 antibiotics were 0.97%, 1.94% and 1.94% respectively (Fig. 2).

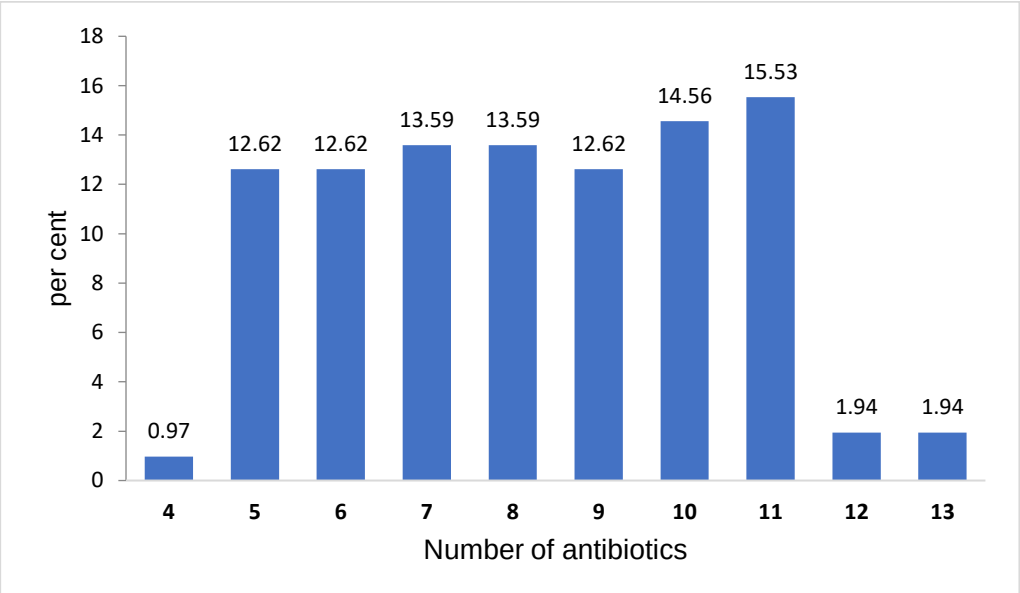

Fig 2: Multidrug resistance in MRSA isolates

β-lactamase production by MRSA strains was detected by an iodometric overlay method. Most MRSA isolates (94.2%) were β-lactamase producer. The percentages of β-lactamase production by in inpatient, outpatient, and HCWs strains were 90%, 95.7%, 96%, respectively (Table 3).

Table2: Resistance of MRSA isolates to different classes of antibiotics.

Antibiotic Class	Antibiotic	MIC range ($\mu g ml^{-1}$)	% of Resistant strains
β-lactamases	Cefoxitin	48->256	100%
	Imipenem	<0.25->256	22.33%
Glycopeptide	Vancomycin	<0.25->256	23.30%
Oxazolidinones	Linezolid	<0.25->256	56.31%
Aminoglycosides	Gentamicin	<0.25->256	76.69%
	Amikacin	<0.25-128	4.85%
Quinolones	Ciprofloxacin	<0.25-16	6.79%
	Levofloxacin	<0.25->256	45.63%
	Lomefloxacin	<0.25-8	8.73%
Tetracycline	Tetracycline	0.5->256	82.52%
Macrolides	Erythromycin	<.25->256	96.11%
	Azithromycin	1->256	94.17%
	Clarithromycin	<0.25->256	56.31%
Folic acid inhibitors	Co-trimoxazole	<0.25->256	55.34%
Fusidane	Fusidic acid	32->256	96.12%

Table 3: β-lactamase production by MRSA isolates obtained from inpatient, outpatient, and health care workers.

Source	β-lactamase producers No (%)	β-lactamase non-producers No (%)
Inpatient (30)	27 (90%)	3 (10%)
Outpatient (23	22 (95.7%)	1 (4.3%)
HCWs (50)	48 (96%)	2 (4%)
Total (103)	97 (94.2%)	6 (5.8%)

MRSA strains were tested for their ability to produce biofilm. All MRSA strains were biofilm producers. A higher percentage of the strains (49.51%) were moderate biofilm producers, compared to the weak (27.18%) and strong (23.30%) producers (Figure 3).

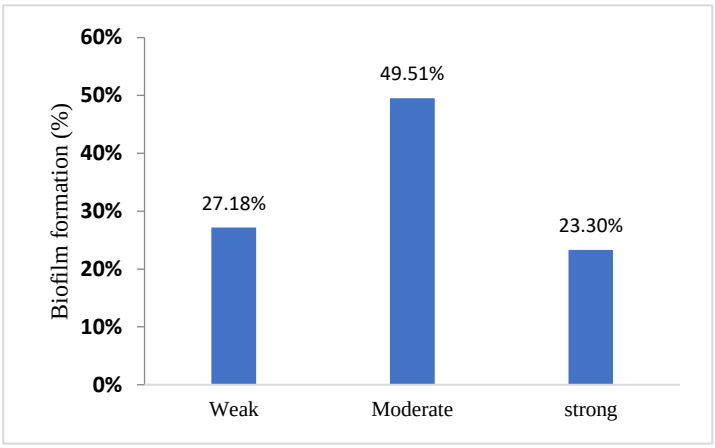

Figure 3: Incidence of biofilm in MRSA isolates.

Discussion

MRSA is a serious human pathogen that causes a wide range of serious illnesses (Cheng, et al., 2010). It is resistant to almost all β-lactam antibiotics and accumulates resistance to many other antibiotics of different classes (Cheng, et al., 2010).

In this study, the overall prevalence of MRSA in S. aureus was 51.5% (103/200), which is high compared to its prevalence in developed countries like the USA and Europe (Shears, 2000; Lee et al., 2018; Turner, et al., 2019). However, a high incidence of resistance to MRSA isolates in Egypt has been reported before (Algammal, et al., 2020).

A relatively high prevalence of MRSA (50.0%) was reported in blood samples (Tan, et al., 2001) which coincide with the higher percentage of MRSA (44.23%) in the blood sample of this study.

A high prevalence of MRSA carriage (70.42%) was detected in HCWs. This contrasts with the carriage rate of MRSA in HCWs reported in other hospitals in Egypt. The prevalence of MRSA in Zagazig, Giza and Al-Azhar University hospitals were 14.6%, 14.8% and 48.8%, respectively (Malek, et al., 2019)

The high carriage rate of MRSA among the HCWs (70.42%) in this study contrasts with its incidence in a previous report in Egypt, where it was 17.02% (Elshabrawy et al., 2017). This suggests that the problem of carriage of MRSA by HCWs is increasing. So, HCWs in Mansoura tertiary hospitals need to be checked regularly for the carriage of S. aureus and MRSA in particular, and they should be immediately decolonised.

MRSA strains were tested for their susceptibility to fifteen antibiotics belonging to nine different classes of antibiotics. More than 80% of the isolates were resistant to four antibiotics (fusidic acid, azithromycin, tetracycline and erythromycin). From 45% to 76% of the isolates were resistant to five antibiotics (linezolid, gentamicin, levofloxacin, clarithromycin and co-trimoxazole). High resist rates to different antibiotics have previously been reported in Egypt (El-Baghdady et al., 2020; Ibrahim et al., 2020).

The percentage of resistance to imipenem (22.33%), in this study, is lower than that previously reported in Egypt in three governorates; Damietta, Dakahlia and Cairo, which ranged between 45 and 78.6% (Taha, et al., 2019; Ibrahim, et al., 2020).

The high levels of resistance of MRSA isolates to most of the antibiotics, in this study, is due to the easy access to antibiotics without a prescription, as well as the excessive and wasteful use of antibiotics in hospitals, animal husbandry, fisheries, and agriculture. The rates of antibiotic resistance in Egypt appears to be a common problem in developing countries (Ahmed, et al., 2014).

In this study, the vancomycin resistance was detected in in 23.4% of the MRSA isolates. Therefore, culture sensitivity testing should be done to guide the use of vancomycin for the treatment of MRSA isolates. Although the rate of vancomycin resistance is comparable to other reports in Egypt (Ibrahiem et al., 2022), it is alarming because this antibiotic is supposed to be the antibiotic of choice for the treatment of MRSA infections and for the treatment of patients allergic to semisynthetic β-lactams (Silva et al., 2021).

Linezolid is a leading antibiotic for the treatment of S aureus infections (Dryden, 2011).The high percentage of resistant strains in this study to linezolid was 56.31%. The incidence of linezolid resistance in progressed countries is usually low. In the USA (Gu, et al., 2013) and Spain (Quiles-Melero, 2014) the reported resistance rates were <2% and 2.8 %, respectively. On the contrary, in the third world countries, reported resistance rates to linezolid have been steadily rising up (Meka and Gold, 2004). In India linezolid resistance ranged between 2–20% % (Gandham, 2014), and in Pakistan it ranged 35 and 48.1% (Azhar, et al., 2017; Wali et al., 2022).

The higher percentage of resistance to linezolid, in this study, in Dakahlia, Egypt is unprecedented and is alarmingly. Currently, linezolid is overprescribed as a drug of choice for the treatment of MRSA infections by physicians of Mansoura university tertiary hospitals (personal communication). This is probably is behind the high detected rate of resistance in this study.

A low percentage of resistance of MRSA isolates to amikacin (56.31%) was detected. This puts it as a good candidate for the treatment of MRSA infections in Dakahlia. However, it should be mentioned that although amikacin displays a potent activity against MRSA, other less toxic agents are preferred and it is usually reserved for the treatment of Gram-negative pathogens (Del Favero et al., 1987).

All MRSA strains were MDR and most of them (95.13%) were resistant to 5 to 11 antibiotics. MDR in MRSA isolates is common in Egypt (Ibrahim, et al., 2020; Ibrahim, et al., 2022) and other countries (Shears and Paul, 2000; Torgersen, et al., 2003).

In this study, the prevalence of β-lactamase production in inpatient, outpatient and HCWs, were 90%, 95.7%, and 96%, respectively. Generally the majority of MRSA strains were reported to harbour a plasmid bearing blaZ and produce active β-lactamase (Hirao, et al., 2012)

All MRSA strains, of this study, were biofilm producers. MRSA strains have been previously reported to form biofilms because most of them possess biofilm-related genes (Silva, et al., 2021).

Conclusion

MRSA strains in tertiary hospitals at Mansoura University, Dakahlia, are highly prevalent and they are highly resistant to different classes of antibiotics. All MRSA strains produced biofilms and most of them produced β-lactamase. The high prevalence of MRSA carriage among HCWs suggests their significant contribution in spreading of MRSA in tertiary hospitals and the community at Mansoura, Dakahlia. This study suggests that MRSA control procedures are currently not consistently followed. Consequently, it is important to implement strict control measure. Patients and HCWs should routinely be regularly screen for their carriage of MRSA and the carriers of MRSA should be decolonized.

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Disclosure

The authors declare that they have no conflict of interest.

Ethical approval

The clinical samples collected were in line with the patient's diagnostic stages and no additional samples were taken. Consent of patients was not required as samples were taken routinely from patients for lab investigations. This research was reviewed and approved by the Ethics Committee of Delta University for Science and technology (FPDU18/2022).

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