

Determination of the Antibiotic Susceptibility Pattern of Clinical Isolates of *Staphylococcus aureus* Collected from Various Diagnostic Centers of Dhaka City, Bangladesh

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Abstract: *Staphylococcus aureus* is one of the leading etiological agents of causing severe skin infections including bacteremia, cellulitis, and different kind of infections. This study was performed to check the drug susceptibility patterns of the phenotypically characterized *S. aureus* isolates collected from some popular diagnostic centers of Dhaka city. A total of 175 non-duplicate isolates were collected from various patients having different age groups and gender. The microorganisms obtained from diverse specimens (i.e. blood, urine, pus, sputum, ear, breast, nipple, catheter, tracheal aspirate) were identified by several phenotypic and biochemical tests. Antibiotic resistance patterns for 22 different antibiotics were evaluated by the Agar-disc-diffusion method to understand antibiotic susceptibility of the isolates. Out of 175, a total of 110 samples were identified as *S. aureus* isolates. The female patients (58%) were likely to be more susceptible to staphylococcal infection than their male counterparts (42%). Of the 110 isolates, most of them were identified as Multi-Drug Resistant (MDR). The highest resistance was recorded against Cefixime (100%), Azithromycin (83.7%), Ciprofloxacin (71%), Levofloxacin (63.6%), and Ceftriaxone (61.8%). Resistance levels of *S. aureus* against other antibiotics were also worrisome. This study highlighted the need for frequent surveillance of antimicrobial susceptibility patterns of *Staphylococcus aureus* with a view of selecting accurate antibiotic therapy in order to avoid the development of drug resistance. However, molecular studies are recommended for developing our current findings.

Keywords: *Staphylococcus aureus*, Skin infections, Multidrug-resistant, Therapy.

I. INTRODUCTION

Staphylococcus aureus is a gram-positive cocci shaped bacterium which is a member of the Staphylococcaceae family and natural human flora, present on the mucous membrane and skin surface and upper respiratory tract [1]. It also causes severe infections such as inflammations of bones, meninges, rashes, and septicemia by penetrating the internal tissues or bloodstream [2-3] as well as various kinds of toxin-mediated diseases such as gastroenteritis, staphylococcal scalded skin syndrome, and toxic shock syndrome. *Staphylococcus aureus* remains an important and frequent cause of morbidity and mortality in tropical countries [4]. *S. aureus* produces various pathogenic factors enabling colonization, adherence, and invasion of the mammary cells of bovine host cells to stimulate intramammary infections. Increased staphylococcal nasal colonization has been reported in insulin-dependent diabetes [5] hemodialysis [6] intravenous drug user [7], patients with symptomatic human immunodeficiency virus infection [8]. *Staphylococcus aureus* can also enter into the body via different kinds of food sources. Staphylococcal Food Poisoning (SFP) is a common foodborne disease globally resulting from different kinds of food consumption containing Staphylococcal Enterotoxin (SE) [9-10]. Symptoms and rapidity of onset (2-8 h) include nausea, vomiting, abdominal cramping with or without diarrhea [11]. Contamination is usually occurred by improper handling of processed food or cooked food, storage conditions allowing the growth of *S. aureus*. It is also a common cause of bovine mastitis and therefore, raw milk sometimes contaminated with *S. aureus* [12]. A total of 386-food borne outbreaks caused by staphylococcal toxins in the European Union according to the recent European Food Safety Authority Report [13].

Staphylococcus aureus has an anomalous ability to acquire resistance against to any antibiotic. Extensive and unplanned use of antibiotics in human and veterinary medicine is the key reason for the emerging of resistant strains of *S. aureus* [14]. Most of the clinically important antibiotic resistance is associated with the transfer of small pieces of extrachromosomal DNA. The mechanisms of resistance are numerous including a large number of plasmid mediate

enzymatic resistance by drug inactivation, antibiotic trapping, efflux pumps, etc [15-16]. The generally used antibiotics for the cure of infections caused by *S. aureus* were β -lactam antibiotics including methicillin. Based on resistance development, *Staphylococcus aureus* can be categorized into two types; Methicillin-Resistant Staphylococcus Aureus (MRSA) and Methicillin Susceptible Staphylococcus Aureus (MSSA) [17]. A small number of Staphylococcal plasmids are known to have the gene for linked antibiotic resistance such as penicillin and fusidic acid, penicillin and erythromycin, penicillin and tetracycline, or neomycin and bacitracin and erythromycin. The most predominant method of penicillin resistance in *Staphylococcus aureus* is the production of penicillinase which destroys the β -lactam ring of penicillin. Intercell contact may be necessary for the transfer of plasmids among cells. So this occurs most often in patients with an infected skin lesion.

The current study provides information regarding the phenotypic characterization of *Staphylococcus aureus* along with the antibiotic susceptibility pattern in the clinically suspected cases of staphylococcal infection from a diverse specimen of various patients.

II. MATERIALS AND METHODS

Collection of samples

The samples were collected between the periods of November 2019 to February 2020. The samples were collected from two diagnostic centers of Dhanmondi and Badda area of Dhaka city, Bangladesh. A total of 175 non-duplicate *S. aureus* isolates were found from blood, urine, pus, sputum, ear, breast, nipple, umbilical, drain tube, catheter, nasal, and tracheal aspirate collected from human patients of different age and gender with different diseases. Sample processing and transportation were maintained as per WHO guidelines [18]. All experiment was performed in the Department of Microbiology, Stamford University Bangladesh.

Isolation and confirmation of *Staphylococcus aureus*

Pure-culture of *S. aureus* was isolated and maintained using Mannitol salt agar (MSA). All the strains were collected aseptically and transferred into Mannitol Salt Agar media. The collected samples were incubated overnight at 37°C for 24 hours and 60 samples were selected for further analysis.

Microscopic analysis

Microscopic analysis of the isolates was done through bacterial size, shape, and staining properties [19]. Initial identification of selected isolates were performed by gram staining procedure, followed by different biochemical test. Cultural and morphological characteristics of selected isolates were identified according to standard microbiological protocols [20].

Biochemical test for the confirmative identification

All isolated bacteria were identified by standard laboratory biochemical tests according to the methods described elsewhere (21). The biochemical tests for *S. aureus* were indole test, MR-VP test, catalase test, oxidase test, urease test, beta hemolysis test, coagulase test, citrate utilization test, H₂S production test as well as mannitol fermentation test [20-22].

Determination of antimicrobial susceptibility by disk diffusion method

Pure culture of *S. aureus* isolated from different clinical samples was selected for assaying antimicrobial susceptibility pattern against a different group of antibiotics such as Amikacin (30 μ g), Amoxyclav (30 μ g), Azithromycin (30 μ g), Cefixime (5 μ g), Ceftriaxone (30 μ g), Cefuroxime (30 μ g), Cephadrine (30 μ g), Ciprofloxacin (5 μ g), Cloxacillin (30 μ g), Cotrimoxazole (30 μ g), Doxycycline (30 μ g), Erythromycin (30 μ g), Fusidic acid (30 μ g), Gentamycin (30 μ g), Meropenem (30 μ g), Teicoplanin (30 μ g), Tetracycline (30 μ g), Vancomycin (30 μ g), Linezolid (30 μ g), Tigecycline (30 μ g), Clindamycin (30 μ g) and Levofloxacin (30 μ g) by Kirby Bauer disc diffusion method [23] as recommended by clinical and Laboratory Standards Institute (CLSI) guideline 2013.

III. RESULTS

In this study, all suspected plates were examined for the presence of *S. aureus* yellow colors on MSA media. All suspected *S. aureus* isolates were confirmed by gram staining microscopic analysis and biochemical tests. Among those 175 samples, 134 strains were isolated from MSA selective media, and 109 of them were identified as *S. aureus* isolates by performing different biochemical tests. Table 1 shows the biochemical test results of all suspected *S. aureus* isolates.

Table 1: Biochemical tests for the isolates bacteria

Assumed Organism	Coagulase Test	H ₂ S production	Indole Test	MR Test	VP Test	Citrate Test	Oxidase Test	Catalase Test	Urease Test	Mannitol Fermentation Test	Nitrate Reduction Test
<i>S. aureus</i>	+	-	-	+	+	-	-	+	+	+	+

MR = Methyl red, VP =Voges-Proskauer,

In this study, a total of 109 isolates were suspected to be suffering from *S. aureus* infections of male and female gender with different ages from 1 to 100 [Figure 1(a)]. We found that female patients are likely to be more susceptible to Staphylococcal infection than male patients. Among the isolates tested 58% of them were obtained from female patients and about 42 % were obtained from male patients [Figure 1(b)].

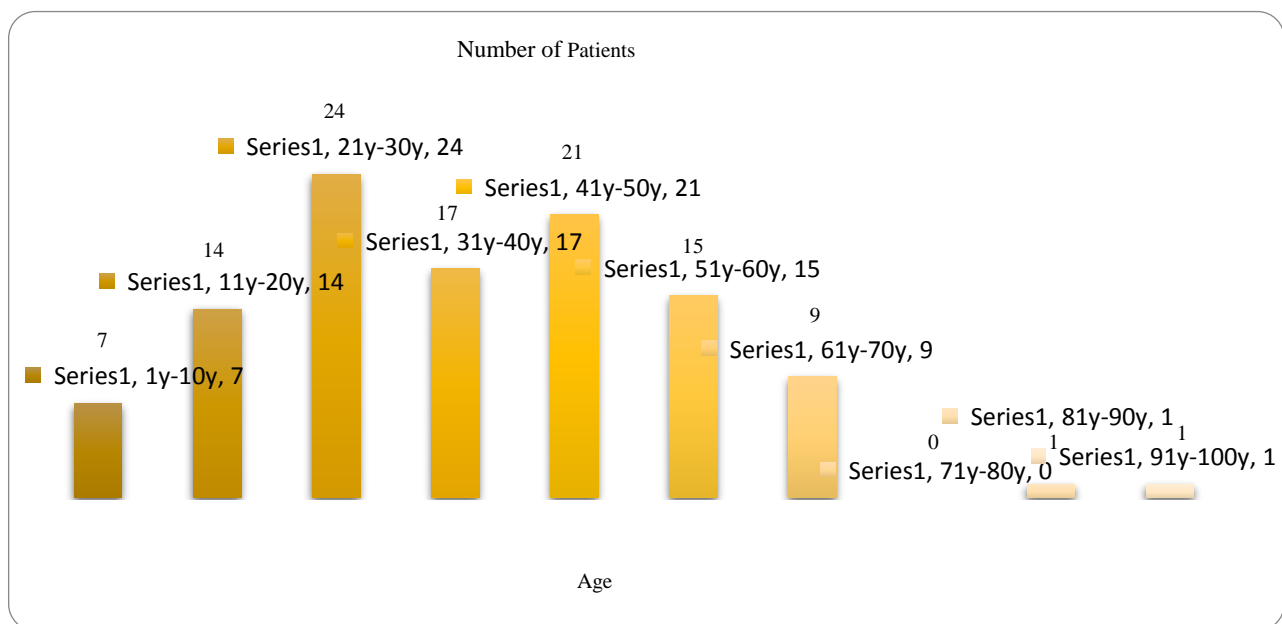


Figure 1(a): Distribution of age groups among respondents

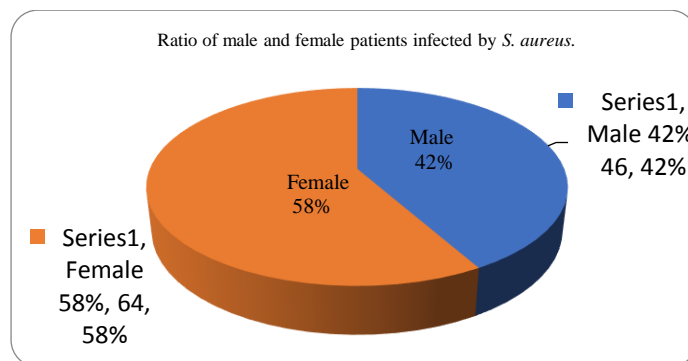


Figure 1(b): Distribution of gender groups among respondents

The rate of infection seems to vary randomly according to age without any selective pattern, although, the tendency of getting affected by different staphylococcal infections seem to be higher in between the ages from 21-50 years old whereas the lowest prevalence rate was observed between the age group from 71-100 years old according to the present study findings.

One hundred and ten clinical isolates of *S. aureus* were subjected to antimicrobial resistance test against different commercial antibiotics. With the 100% resistance of cefixime demonstrated as the most resistant antimicrobial followed by Azithromycin (83.7%), Ciprofloxacin (71%), Levofloxacin (63.6%), Ceftriaxone (61.8%), Cloxacillin (34.5%), Cotrimoxazole (33.6%), Cephadrine (31%), Clindamycin (29.1%), Clindamycin (29.1%), Cefuroxime (27.3%),

Meropenem (23.6%), Gentamycin (22.7%), Fusidic Acid (21.8%), Amoxyclav (19.1%), Amikacin (9.1%), Tigecycline (3.6%), Linezolid (2.7%). However, Teicoplanin (0%), Vancomycin (0%) was found to be the most effective drug as all the studied isolates were proved to be sensitive against this antibiotic [Figure 2].

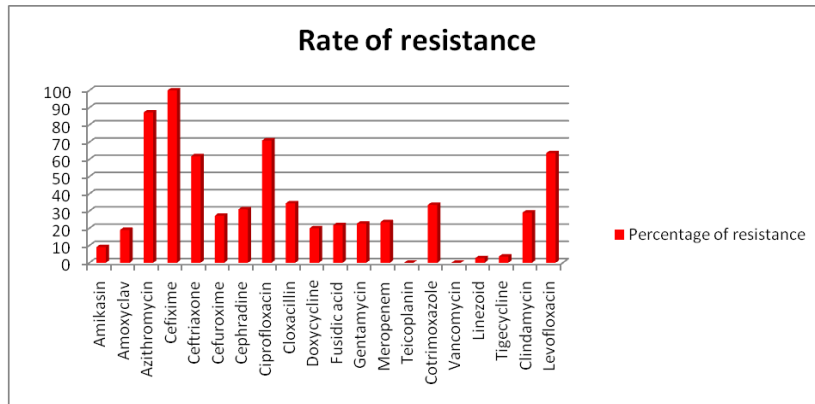


Figure 2: Antibiotic resistance pattern of *S. aureus* isolates (n=110)

In our present study, 85 strains (77%) were found to be multidrug-resistant among 109 *Staphylococcus aureus* isolates. Table 2 shows the various sources of specimen for the isolation of *Staphylococcus aureus* and antibiotic resistance pattern of different isolates to various antibiotics among age groups and sex. The highest frequency of resistance was from (61-70) years old in case of male patients 16 (80%) and observed with Pus, Right ear and Wound swab infections while the least resistance was in the (81-90) years old in case of male 3 (15%) & female patients 4 (20%) and observed with Urine infections. Interestingly, all antibiotics were proved to be effective for the age group (71-80) years old and (91-100) years old patients.

Table 2: Antibiotic susceptibility patterns of *Staphylococcus aureus* isolates

Age group (Years)	Sex	Specimen type	Number of antibiotics and percentage of resistance
1-10	M	Umbilical swab	13(65%)
	F	Blood, Pus	12(60%)
11-20	M	Pus	5(25%)
	F	Urine, Blood, Wound swab, Right ear	9 (45%)
21-30	M	Right ear, Catheter tip, Pus, Nasal swab	10 (50%)
	F	Urine, Nipple, Wound swab	10 (50%)
31-40	M	Blood, Pus	9 (45%)
	F	Urine, Breast, Pus, Wound swab	11 (55%)
41-50	M	Pus, Wound	14 (70%)
	F	Tracheal Aspirate, Blood, Pus, left ear, Catheter tip, Wound swab	13 (65%)
51-60	M	Urine, Wound swab	15 (75%)
	F	Blood, Drain tube	11 (55%)
61-70	M	Pus, Right ear, Wound swab	16 (80%)
	F	Blood, Blood catheter, Pus	11 (55%)
71-80	M	-	-
	F	-	-
81-90	M	Urine	3 (15%)
	F	Urine	4 (20%)
91-100	M	-	-
	F	-	-

IV. DISCUSSION

In our present study, female patients were more affected (58%) than male patients (48%). Although a staphylococcal infection can occur at any age, in our present study, the patient's age ranged from 21-30 years were more affected in terms of male and female patients whereas the lowest incidence rate of infection was in between 81-100 years of age.

The patient's age ranged from 21-30 years showed the highest degree of susceptibility pattern [24] for the staphylococcal infection followed by 41-50 years (21), 31-40 years (17), 51-60 years (15), 11-20 years (14). Although, no infected patients were found in the age ranged from 71 to 80 years after investigating 110 clinically suspected patients.

Staphylococcus aureus develops resistance very quickly to different antibiotics over a period of time. Due to the reckless and widespread use of antibiotics, the resistance profile of microorganisms is changing gradually, especially in developing countries [24-25]. In our study, antibiotic resistance pattern revealed that among the isolates, 100 % isolates showed resistance against Cefixime, followed by Azithromycin (83.7%), Ciprofloxacin (71%); intermediate level of resistance was showed in case of Levofloxacin (63.6%), Ceftriaxone (61.8%). The least level of resistance was found against Tigecycline (3.6%), Linezolid (2.7%). In our present study, Vancomycin (0%) was found to be the most effective drug, this outcome is in similar with the findings of Hizbullah et al. (2015) [26]; Shah et al. (2016) [27]; Ullah et al. (2016) [28]. All of these studies had reported 0% resistance against vancomycin. However, resistance against Ciprofloxacin and Gentamycin was estimated as 71% and 22% respectively which is almost similar to a recent study [29] where Ciprofloxacin and Gentamycin were encountered as 77% and 13.21% respectively. The frequency of antimicrobial resistance was observed with different ages and gender in terms of different specimen types. The highest frequency of resistance was from (61-70) years old male patients 16 (80%). However, non-resistance antibiotic was found in the age between (71-80) years and (91-100) years old patients.

The susceptibility pattern of antimicrobials against *Staphylococcus aureus* has given us an outline of the uncontrolled use of antibiotics through this study. The judicious use of antibiotics by the experts such as doctors or health professionals and effort to control misuse of antibiotics and procurement will assist in limiting the increasing rate of antibiotic resistance in the pathogenic microorganisms. Inappropriate prescribing of antibiotics for viral infections, against which they have no effect or antibiotic use for these conditions are unnecessary. Rational drug policy should be in use before addressing the potent antibiotics to the country [30]. Antibiotic administration should follow certain minimal requirements [31].

In Bangladesh, it is necessary to carry out a constant assessment of antibiotic susceptibility patterns of resistant pathogenic microorganisms for commonly used antimicrobial agents in a particular environment. The present situation of antimicrobial resistance in Bangladesh should be taken into account seriously, otherwise, it will become an uncontrollable problem not only in Bangladesh but also worldwide in the near future.

V. CONCLUSION

Staphylococcus aureus remains a worldwide cause of infection of skin as well as causing life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis specially on developing and underdeveloped countries like Bangladesh [32]. In conclusion, our study emphasizes the development of public awareness regarding the prevention and spread of staphylococcal infection. The best way to prevent the spread of *S. aureus* in hospital settings is to screen health caretakers for the presence of these organisms. Continuous monitoring of the antimicrobial susceptibility pattern of *S. aureus* isolates for the selection of appropriate therapy is also very much required. However, further molecular studies are recommended to study and monitor the epidemiology of multiple drug-resistant *S. aureus*.

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