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Effect of SDS on Plasmid Isolation and Antimicrobial Resistance Pattern of Clinical Isolates of *Staphylococcus Aureus*

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Abstract

In the present study antimicrobial resistance pattern of 32 clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) to 17 antimicrobial drugs was done from which 7 multidrug resistant isolates were selected. Amongst the 32 isolates of *Staphylococcus aureus* 21(66%) were found resistant to amoxicillin, 19(59%) to cloxacillin, 18(56%) to oxacillin, 32(100%) to penicillin G and most surprisingly 15(53%) to vancomycin. They showed maximum (91%) sensitivity to both gentamicin and imipenem, 84% to tetracycline and 81% to ciprofloxacin; moderate sensitivity to neomycin (78%) and rifampicin (75%); low sensitivity to ceftriaxone and chloramphenicol (66%) and erythromycin (53%). Sensitivity showed less than 50% to cephalexin, cephradin, cotrimixazole and methicillin group antibiotics. In this study 1% SDS with acridine orange was used to isolate plasmids from the clinical isolates. Isolates showing resistance to an antimicrobial drug became sensitive to the same antimicrobial when grown in luria broth in

presence of 1% SDS and 1% SDS plus acridine orange. The change in sensitivity pattern was to ceftriaxone, cephalexin, cephradine, cotrimoxazole, gentamicin, neomycin, and tetracycline. After gel electrophoresis, plasmid profiles of the isolates showed that those isolates grown in presence of 1% SDS and 1% SDS plus acridine orange were more prominent than those without SDS treatment.

Keywords: MRSA, resistance, sensitivity, SDS, acridine orange, plasmid.

1. Introduction

According to the Infectious Diseases Society of America in the January 2009 highlighted the impact of the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) as a group of particularly troublesome bacteria having the ability to "escape" the effects of current antimicrobial agents (Boucher *et al*., 2009). Drug resistance is an alarming problem worldwide and it is spreading rapidly due to overuse, self medication, and non-therapeutic use of antimicrobials (Slama *et al*., 2005).

Multiple antimicrobial resistances of the bacterial pathogens are of great concern both in veterinary and human medicine worldwide (Aryal, 2001). Antimicrobial resistance is a serious problem in the treatment of animal and human patients with infectious diseases. Like other bacterial pathogens *Staphylococcus aureus* has become resistant to many antimicrobials through the acquisition of mobile drug resistant genes. A major force in the over expression of endogenous multidrug transporters and spread of plasmid encoded multidrug transporters in the huge consumption of antibiotics in human therapy, animal husbandry and agriculture (Islam *et al*., 2008). *Staphylococcus aureus* is a major cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling medical devices. *Staphylococcus aureus* is responsible for variety of suppurative (pus-forming) infections and toxinoses in humans. It causes superficial skin lesions such as boils, styes and furuncules or impetigo; more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis and endocarditis (Todar, 2008). Hospital acquired infection is often caused by antibiotic resistant strains and can only be treated with vancomycin or an alternative. Methicillin-resistant *Staphylococcus aureus*, or MRSA strains have recently emerged outside the hospital becoming known as community associated- MRSA or CA-MRSA or superbug strains of the organism, which now account for the majority of staphylococcal infections seen in clinics. Many of the community associated staphylococcal infections are now methicillin resistant (Todar, 2008; Islam *et al*., 2008).

The purpose of this study is to find whether the drug resistant gene was present in plasmid or not and to determine the correlation of antimicrobial resistance pattern with their plasmid profile**.** The effect of sodium dodecyl sulphate (SDS) and acridine orange on plasmid isolation has also been studied to determine the antimicrobial resistance pattern of multidrug resistant clinical isolates of *Staphylococcus aureus*.

2. Materials and Methods

2.1. Collection of Isolates

Thirty two isolates of *Staphylococcus aureus* were collected from different diagnostic centres of Dhaka, Bangladesh. The samples were collected from pathological specimen of urine, pus, blood, high vaginal swabs and wound swabs. The isolates were identified on the basis of their colony morphology, microscopic morphology and biochemical tests for reassurance and reconfirmation of the strain.

2.2. Antibiotic Suseptibility Test

The antibiotic sensitivity pattern of *Staphylococcus aureus* to 17 antibiotics namely amoxycillin (Am 30µg), ceftriaxone (Ci 30µg), cephalexin (Cp 30µg), cephradine (CV 25µg), cholramphenicol (C 30µg), ciprofloxacin (Cf 5µg), cloxacillin (CX 1 µg), cotrimoxazole (CO 25µg), erythromycin (E 15µg), gentamycin (G 10µg), imipenem (I 10µg) neomycin (N 30µg), oxacillin (OX 1µg), penicillinG (P 10µg), rifampicin (R 5µg), tetracycline (T 30µg) and vancomycin (VA 30µg) was determined by disc diffusion Kirby Bauer (Bauer *et al*., 1966) method as per recommendation of National Committee for Clinical Laboratory Standard (NCCLS, 1997).

2.3. Antibiotic Susceptibility Test of *Staphylococcus Aureus* in the Presence of 1% SDS Solution

From thirty two isolates, 7 isolates of *Staphylococcus aureus* were selected and cultured without SDS, with of 1% SDS and 1% SDS plus acridine orange. SDS solution is used as the curing agent. Antibiotic susceptibility test for these 7 samples was carried out in absence of SDS, in presence of 1% SDS and 1% SDS plus acridine orange by disc diffusion Kirby Bauer (Bauer-Kirby., 1996) method using the same 17 antimicrobials. Then plasmid profile analysis of the above sample was done using gel electrophoresis plasmid isolation. To isolate plasmid DNA from bacteria a scaled-up miniprep followed by additional purification was done. This resulted in relatively large amount (several micrograms) of very pure plasmid DNA.

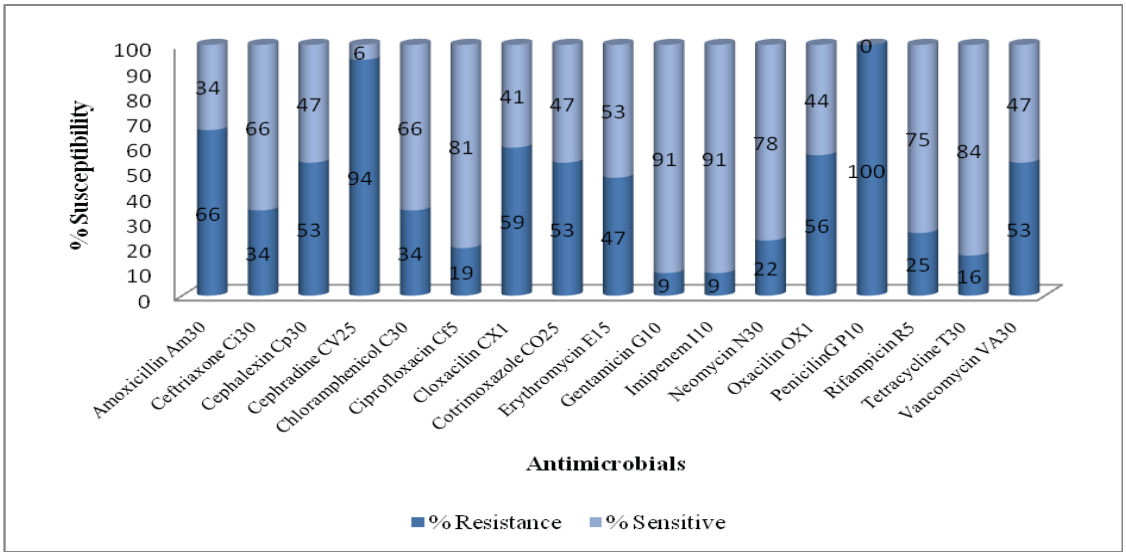
2.4. Separation of Plasmid DNA by Agarose Gel Electrophoresis

Electrophoresis was carried out in a horizontal 1.5% agarose gel. The gel was viewed on an ultraviolet (UV) light box (short wave, ultraviolet products, Inc., San Gabriel, California, USA, 254nm). The photographs were taken under UV illumination using sony cyber shot 5.1 megapixel, USA.

3. Results

The results of antimicrobial susceptibility of *Staphylococcus aureus* to 17 antibiotics are represented in Figure 1, where percentage of susceptibility is taken in the y-axis and different antimicrobial agents are taken in the x-axis. The antibiotic susceptibility pattern test was carried out with selective seven samples without SDS, in the presence of 1% SDS and with 1% SDS plus acridine orange (Table 1).

Figure 1: Antimicrobial susceptibility pattern of 32 clinical isolates *Staphylococcus aureus*



3.1. Gel Electrophoresis

The plasmid profile analysis indicates that plasmid DNA was absent without SDS treatment. But after culturing the multi drug resistant isolates in 1% SDS in luria broth the plasmid profile showed the presence of plasmid (Figure 2). The appearance of plasmid was more prominent when cultured with 1% SDS plus acridine orange in luria broth. As expected, the sensitivity of isolates to the above fourteen antimicrobials has increased significantly. But the plasmid profile shown in the gel electrophoresis photograph indicates that even by liquid culture with 1% SDS, plasmid remained inside bacteria.

Table 1: Antimicrobial susceptibility pattern of *Staphylococcus aureus* after liquid culture

Sample No.	Specimen	Amoxicillin Am30	Ceftriaxone Ci30	Cephalexin Cp30	Cephradine CV25	Chloramphenicol C30	Cloxacilin CX1	Cotrimoxazole CO25	Erythromycin E15	Gentamicin G10	Imipenem I10	Neomycin N30	PenicilinG P10	Rifampicin R5	Tetracycline T30	
1	SA Rt. Eye 227	R	S	S	S	S	R	S	S	S	S	S	R	S	S	Grown in the absence of SDS
2	SA PUS 801M	R	R	R	R	S	R	R	R	S	S	R	R	S	R	
3	SA 29/F W/5	R	S	S	R	S	R	R	S	S	S	R	R	S	S	
4	SA 29Y/M	R	R	R	R	S	R	S	S	S	S	S	R	S	R	
5	SA PUS 20/M	R	R	R	R	S	R	R	R	S	S	S	R	S	R	
6	SA9D/M U/S	R	R	R	R	S	R	R	S	R	S	R	R	S	R	
7	SA C-72 PUS	R	S	R	R	S	R	S	S	S	S	R	R	S	S	
1S	SA Rt. Eye 227	R	S	S	S	S	R	S	S	S	S	S	R	R	S	Grown in presence 1% SDS
2S	SA PUS 801M	R	S	S	R	S	R	S	R	S	S	S	R	R	S	
3S	SA 29/F W/5	R	S	S	R	S	R	S	S	S	S	S	R	R	S	
4S	SA 29Y/M	R	S	R	R	S	R	S	S	S	S	S	R	S	S	
5S	SA PUS 20/M	R	S	S	S	S	R	S	S	S	S	S	R	R	S	
6S	SA9D/M U/S	R	S	S	S	S	R	S	S	S	S	S	R	R	S	
7S	SA C-72 PUS	R	S	S	R	S	R	S	S	S	S	S	R	R	S	
1AS	SA Rt. Eye 227	R	S	S	S	S	R	S	S	S	S	S	R	S	S	Grown in presence of 1% SDS plus acridine
2AS	SA PUS 801M	R	S	S	R	S	R	S	R	S	S	S	R	R	S	
3AS	SA 29/F W/5	R	S	S	R	S	R	S	S	S	S	S	R	R	S	
4AS	SA 29Y/M	R	R	R	R	S	R	S	R	S	S	S	R	R	S	
5AS	SA PUS 20/M	R	S	S	S	S	R	S	R	S	S	S	R	R	S	
6AS	SA9D/M U/S	R	S	S	S	S	R	S	R	S	S	S	R	R	S	
7AS	SA C-72 PUS	R	S	S	R	S	S	S	S	S	S	S	R	R	S	

4. Discussion

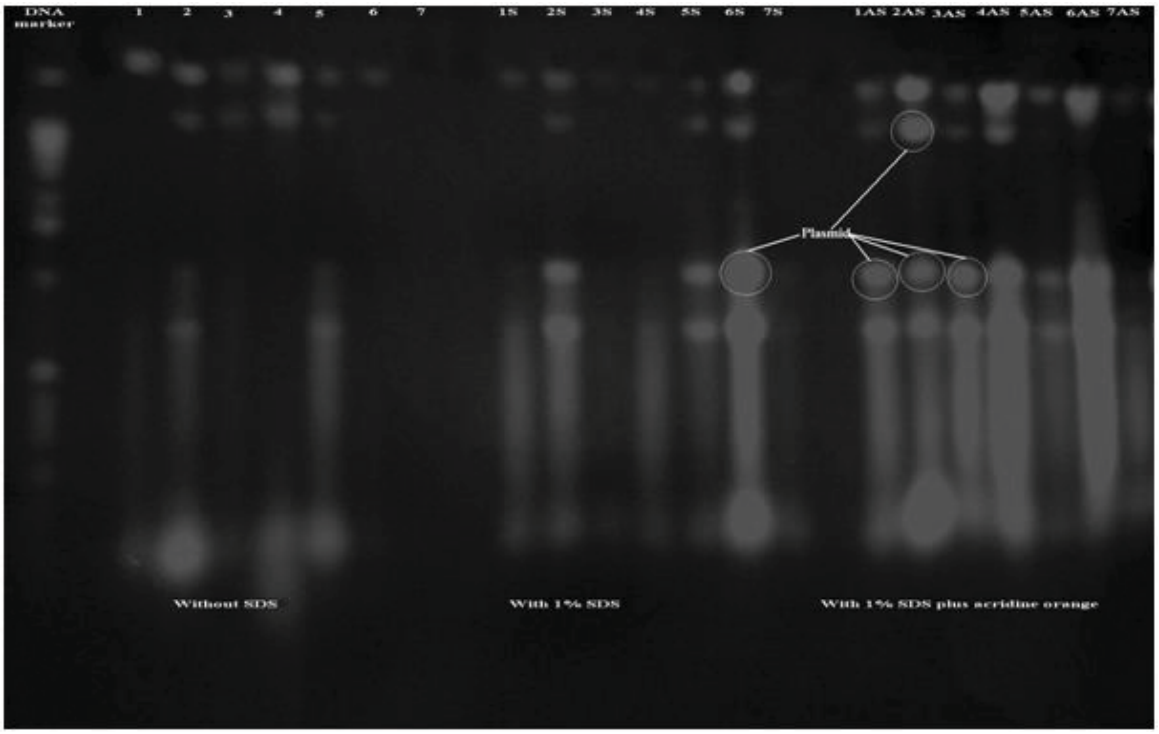
Resistance to antimicrobial is highly prevalent in bacterial isolates worldwide, particularly in developing countries. Like other bacterial pathogens *Staphylococcus aureus* has become resistant to many antimicrobials through the acquisition of mobile drug resistant genes.

In our study, it was observed that the isolates of *Staphylococcus aureus* from different pathological specimen showed different degree of sensitivity to different antimicrobials. Seventeen

different antimicrobial were used to test susceptibility. Most of the isolates of this study were resistant to the first line antibiotics that are commonly prescribed by the physicians.

Although from Figure-1 it has been postulated that most of the isolates are multidrug resistant and after culturing the isolates in 1% SDS in luria broth the plasmid profile showed the presence of plasmid (Figure-2). The appearance of plasmid was more prominent when cultured with 1% SDS plus acridine orange in luria broth.

Figure 2: Plasmid profile of the 7 selected multidrug resistant isolates of *Staphylococccus aureus* in absence and in presence of 1% SDS, and with 1% SDS plus acridine orange



However, the presence of plasmid has been found in the plasmid profile of all three experimental conditions of without SDS, 1% SDS and with 1% SDS plus acridine orange. The isolate 3 showed resistance to cotrimoxazole and neomycin but the same isolate was sensitive when grown in presence of 1% SDS and 1% SDS plus acridine orange. Same was the case with few other isolates with other antimicrobials, like isolate 4 to tetracycline; isolate 5 to ceftriaxone, cephalexin, cephradin, cotrimoxazole, and tetracycline; isolate 6 to ceftriaxone, cephalexin, cephradin, cotrimoxazole, gentamicin, and tetracycline; isolate 7 to cephalexin, and neomycin. So it is evident that in sample number 3-7 the resistant gene for the respective antibiotics may be present in the plasmids.

5. Conclusion

Plasmids may be responsible for the resistance to most of the first line the antibiotics, however those plasmid did not appear in the gel electrophoresis photograph of isolates cultured without 1% SDS or 1% SDS plus acridine orange. Appearance of plasmids responsible for sensitivity to some antibiotics in presence of 1% SDS or 1% SDS plus acridine orange of this study, however, doesn't propose any cause or correlation between antibiotic resistance of *Staphylococcus aureus* and plasmid of the isolates. So, further studies may be necessary to confirm this issue in future. If the gene responsible for the multidrug resistance can be located, genetic engineering and further research can be done to prevent *Staphylococcus aureus* becoming resistant.

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