Isolation of DNA Plasmid from Antibiotic-Resistant *Staphylococcus aureus*

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***Abstract*—*Staphylococcus aureus* is an opportunistic organism responsible for most nosocomial infection. From a total of 100 staphylococci isolates, 41 were *S. aureus* collected from 8 (19.5%) tonsillitis, 9 (22%) skin, 13 (31.7%) wound, 6 (14.6%) nose, and 5 (12.2%) ear infection samples. All bacterial isolates were identified by the biochemical, cultural, and microbial characteristics confirmed by Api Staph System. β-lactamase test of *S. aureus* revealed that 21 isolates were positive. While urease test was positive in 31 (75.61%) of the isolates. The sensitivity test of four quinolone groups showed that 50.2% isolates were resistant to norfloxacin, 44.1% were resistant to ofloxacin, and 39.8% were resistant to ciprofloxacin, whereas the lowest resistance was 25.7% to levofloxacin. The β-lactamase-positive *S. aureus* showed a high resistance in comparison to that β-lactamase-negative isolates. The range of the minimum inhibitory concentrations of levofloxacin was 16-512 µg\mL. A single plasmid was detected in two isolates with the same size. The DNA plasmid was determined from levofloxacin-resistant isolates.**

***Index Terms*—Antibiotic resistance, Levofloxacin, Plasmid, *Staphylococcus aureus,* β-lactamase.**

I. Introduction

Staphylococcus aureus is a normal flora, causative for a wide range of chronic disease, and difficult to control due to tolerant of a high concentration of antibiotics. It can become resistant to antibiotics by horizontal transfer to genes, chromosomal mutation, and antibiotic selection [1,2]. 30% of humans are asymptomatic nasal carriers [3]. In human, antibiotic resistance depends on the region, infection site, and and mode of disease transmission [4].

Microbial flora staphylococcal species are without mecA or mecC gene, and these genes are blocked binding β-lactam antibiotics and located on a mobile genetic island called staphylococcal cassette chromosome mec (SCC mec), until 1940s, when penicillin G was used for treating staphylococci infection, first antibiotic resistance acquired by penicillinase plasmid [5]. The mec gene can be transmitted between *S. aureus* strains and between other *Staphylococcal* species [6]. Mortality rate of *S. aureus* bacteremia reach to 20–40% [7] 20% of the *S. aureus* genome are mobile genetic elements, such as plasmids. In addition to antimicrobial resistance, plasmid can also be contributed in pathogenesis, virulence, and host adaptation [8]. Today, many antibiotics are used for *S. aureus* infection treatment such as flucloxacillin, dicloxacillin, cephalosporins (cefazolin, cephalothin, and cephalexin), clindamycin, lincomycin, erythromycin, vancomycin, teicoplanin, combination of rifampicin and fusidic acid, lincosamides (clindamycin and lincomycin), or cotrimoxazole. Linezolid and quinupristin/dalfopristin also are used [9]. The aim of this study is to examine *S. aureus* for susceptibility to several antimicrobial agents and plasmid content and produce enzymes β -lactamase.

II. Materials and Methods

A. Samples Collection

A total of 100 isolate *Staphylococcus* spp. were isolated from different clinical samples, tonsillitis, ear, nose, skin, and wound infection at several hospitals in Baghdad.

The cultures were characterized microscopically by Gram's staining, biochemically, and microbial characteristics by Api Staph System [10].

B. Production of β-lactamase Enzyme Test

Production of β-lactamase enzyme was performed by rapid iodometric method [11].

C. Production of Urease Enzyme Test

Urease enzyme test was performed by inoculating the bacteria onto the tube containing urea agar slant and incubated at 37°C for 18–24 h [11].

D. The Sensitivity Test to Four Quinolones Groups

Antibiotic susceptibility test was performed using disk diffusion method on Mueller-Hinton Agar according to the National Committee of Clinical Laboratory Standards.

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Antibiotics included ofloxacin, norfloxacin, ciprofloxacin, and levofloxacin [12].

E. E. Determination of the mnimum inhibitory concentrations of levofloxacin

Titration method was used for determining levofloxacin minimum inhibitory concentrations (MICs) [13].

F. Plasmid DNA Extraction

Plasmid DNA was extracted from resistant bacteria for norfloxacin using the standard method and alkaline sodium dodecyl sulfate method with Promega DNA kit.

Results and Discussion

A. Identification of S. aureus

Of 100 isolates, 41 were *S. aureus,* while 59 were *Staphylococcus* spp. from different clinical sources as shown in Table I. Endalafer, *et al.*[14] mentioned that *S. aureus* is a commensal or normal flora on the skin and [15] reported that these organisms are common contaminants of wounds and can migrate from the urinary, respiratory, and gastrointestinal tract.

B. Production of β-lactamase Enzyme

Of 41 isolates, 21 (51.2%) isolates produce β-lactamase. The antibiotics resistance of *S. aureus* depends on the amount of β-lactamase secreted [16].

TABLE I Isolated Bacteria According to Source and the Percentage of Infection

Source	*Staphylococcus* spp.	*S. aureus*	
	n (%)	*n* (%)	Total
Tonsillitis	10 (16.9)	8 (19.5)	18
Swab of Skin	7 (11.9)	9 (22)	16
Wound swab	20 (34)	13 (31.7)	33
Nose swab	8 (13.5)	6 (14.6)	14
Ear swab	14 (23.7)	5 (12.2)	19
Total	59	41	100

Staphylococcus aureus: S. aureus

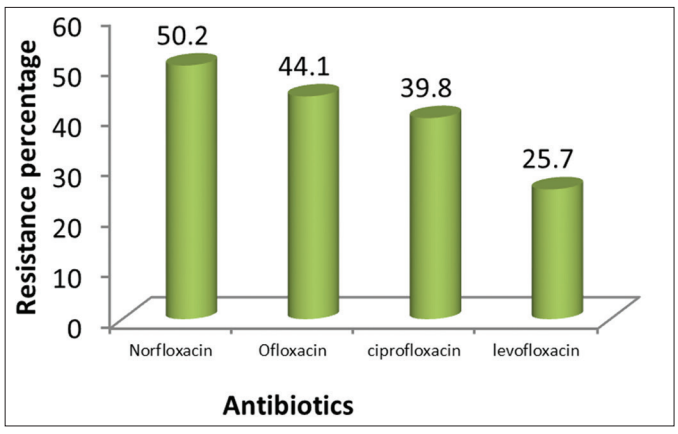

Fig. 1. *Staphylococcus aureus* quinolone-resistant percentage.

C. Production of Urease Enzyme

This study showed that 31 (75.61%) isolates were produced *urease,* whereas 10 (24.39%) isolates were not produced. Mobley, *et al.* [17] reported that *S. aureus* uses urease activity to protection against low PH.

Urease was produced by 18.1% of coagulase-negative staphylococci isolates [18], and according to Longauerova [19] study showed urease production has been observed to be an important virulence factor in *Staphylococcus simulans*, *Staphylococcus capitis*, *Staphylococcus hominis*, *Staphylococcus warneri*, and *Staphylococcus caprae* [19].

D. Antibiotic Resistance

Among 41 *S. aureus* isolates, 50.2% were found to be resistance toward norfloxacin, 44.1% were resistant to ofloxacin, and 39.8% were resistant to ciprofloxacin, while the lowest resistance was 25.7% to levofloxacin (Fig. 1).

Rosato, *et al.* [20] study found that levofloxacin was the most commonly fluoroquinolone used in hospital followed by ciprofloxacin, whereas ciprofloxacin was used most often in community followed by levofloxacin. A significant relationship was found between fluoroquinolone use in hospitals and the percentage of *S. aureus* isolates that were methicillin-resistant *S. aureus* (MRSA) [21].

The percentage of isolate β-lactamase positive was 75% to levofloxacin, 63.5% to ciprofloxacin, 57.2% to ofloxacin, and 53.8% to norfloxacin, while percentage of negative isolates to β-lactamase was 25% to levofloxacin, 36.5% to ciprofloxacin, 42.8% to ofloxacin, and 46.2% to norfloxacin (Fig. 2). *S. aureus* with β-lactamase enzyme had higher resistant than negative isolate to β-lactamase enzyme. *Staphylococci* have resistance to b-lactam antibiotics by producting β-lactamase enzymes through acquisition of the mecA gene [20], and more than 95% of staphylococcal isolates have β-lactamase [8], in North America, Europe, and Asia 25–50% of clinical isolates are MRSA [22].

β-lactamase *S. aureus* isolate activity is a higher percentage than other staphylococci, and this result agrees with Robles, *et al.* [23]. Saini, *et al.* [24] mentioned in his study the relationship between antimicrobial use and antimicrobial resistance.

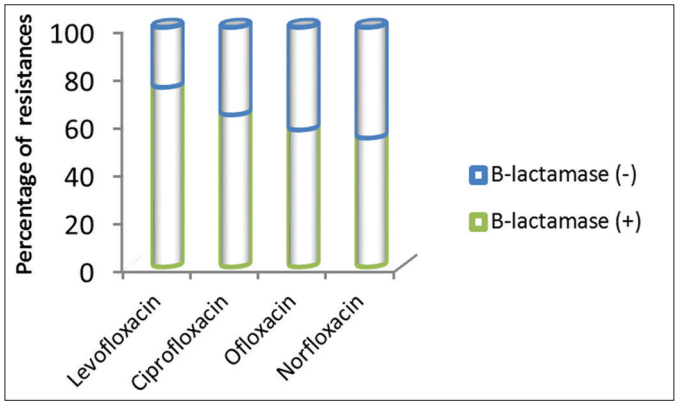

Fig. 2. Percentage of resistant antibiotic for positive and negative isolate β-lactamase.

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The MRSA strains that had SCCmec genes are resistant to β-lactam antibiotics [25].

E. Determination of MIC to Levofloxacin

MIC of levofloxacin to *Staphylococcus* isolates was between 16 and 512 mg/mL, and there were 5 isolates that resistant of 512 mg/mL (Table II). These results are compatible with the study of Islam [26]. *S. aureus* isolates were hyperresistant to levofloxacin, and the differences in the levofloxacin MICs value may be due to differences in clinical isolated source and type of antibiotics were used for treatment.

F. Plasmid DNA Extraction of S. aureus

Plasmid DNA extraction was isolated from resistant bacteria to levofloxacin, and the extraction results showed the presence of single plasmid bands in two isolates (P_{19} and P_{34}) as shown in Fig. 3. Plasmid-mediated quinolone resistance encodes for qnr genes that protect DNA gyrase and topoisomerase IV from quinolone inhibition [27].

qnr genes are usually found in multiresistance plasmids linked to other resistance determinants like β-lactamase genes [28,29].

qnr genes are acquired from mobilizing or transposable elements on plasmids found in clinical and environmental isolates around the world and appear to be spreading [27].

TABLE II MIC OF ISOLATES TO LEVOFLOXACIN

NO isolate	MIC µg/mL	NO isolate	MIC µg/mL
9	128	14	64
10	32	16	16
46	32	18	64
67	512	19	512
90	32	32	256
87	16	43	32
5	64	24	16
15	32	54	64
1	128	33	512
6	128	34	512
39	512	59	32
41	32	36	64

MIC: Minimum inhibitory concentrations

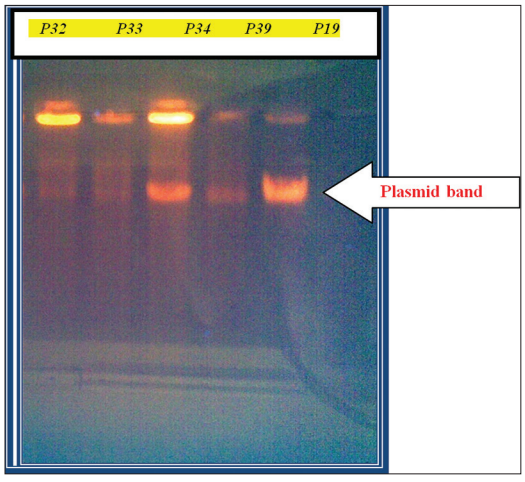

Fig. 3. *Staphylococcus aureus* isolate DNA plasmid resistant to levofloxacin on agoras gel electrophoresis (0.8%).

IV. CONCLUSION

Staphylococcal spp. antibiotic resistance wide spreading can be acquired from other bacterial genus or species, especially in hospital or from environment.

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