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Prevalence of IS256 in Multidrug Resistance *Stphylococcus aureus* Isolated from Clinical Samples

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Abstract: ***Staphylococcus aureus is a major human pathogen and has been able to acquire resistance to nearly all clinically used antibiotics. This study was conducted to investigate the genetic organization of IS256 in clinical isolates of S. aureus. A total of 22 isolates of S aureus from nosocomial infections were collected, identified, and examined to determine the distribution of IS256 residing in genomic DNA of these isolates. Results showed that 18(82%) of these isolates are multidrug resistance, the genomic DNA of also 18 isolates carry copies of the insertion sequence IS256. Moreover, detection of IS256 was found to be associated with antibiotic resistance in these clinical isolates. The data suggest that IS256 is a characteristic element in the genome of multiresistant nosocomial S. aureus isolates.***

1. Introduction

Staphylococcus aureus as a causative organism of nosocomial infection is now often multidrug resistant causing acute and chronic infections resulting in significant morbidity, it causes acute and chronic infections resulting in significant morbidity (1,2). The appearance of antibiotic resistance among bacterial pathogens is a major problem in treatment of infectious disease in both community and in healthcare settings throughout the world (3). *S. aureus* has acquired resistance to nearly all antibiotics used in clinical practice. Whereas some resistance mechanisms are conferred by uptake of resistance genes, others evolve by mutation (4).

IS*256* is an insertion sequence widespread in the genomes of multiresistant enterococci and staphylococci (5). The element, which is 1,324 bp in size, consists of a single open reading frame encoding a transposase protein flanked by noncoding regions (NCRs) harboring imperfect inverted repeats (IRs). IS*256* occurs in multiple free copies in its host genomes but is also known to form the ends of composite transposon Tn*4001* conferring aminoglycoside resistance (6). In *Staphylococcus epidermidis*, IS*256* has been identified as a typical marker of hospital-acquired multiresistant and biofilm forming clones causing opportunistic infections in immunocompromised patients (7). IS*256* has been detected in the genome of several clinical isolates of *S. aureus* in multiple copies, alone or flanking both ends of the amino -glycoside resistance transposon Tn*4001*. Tn*4001* is composed of 1.9 Kb central region flanked 1.3 Kb (1324 bp) inverted repeats (5). The IRs flanking the resistance determinant of Tn*4001* has been designated insertion sequence*256*. Tn can transpose by a copy and paste mechanism (6). Gene inactivation is possible effects of IS transposition in the function and expression of a target gene many cases have been described illustrating the modulation of resistance, virulence and metabolic activities by IS-mediated gene inactivation (7).

The observation that some of these genetic processes occurred by the action of insertion sequence elements prompted us to investigate the distribution of common Staphylococcal IS elements among *S.aureus* isolates of clinical origin , aim the search analyzed the relationship between IS presence, antibiotic resistance (8). In this study, PCR screening was used to detect IS*256* in *S.aureus* from nosocomial infections and analyzed the relationship between the presence of IS*256* and antibiotic resistance.

2. Materials and Methods

Bacterial isolates

A total of 109 swab samples were collected from urine, boil, wounds, nasal, vagina, blood, and sputum for patients with wide range of ages attending the division of Microbiology/National center for educational Laboratories in the Medical City and the division of Microbiology Laboratories/ Private Nursing Hospital in Baghdad during the period from July to September/2019. From these samples, 22 isolates were identified as *S.aureus* according to their cultural, morphological and biochemical tests (Gram staining, coagulase, catalase, oxidase, type of hemolysis, urease, lecithinase, methyl red, Vogues Proskaure, citrate utilization tests, and then full identification by Vitek-2 system.

Antibiotic susceptibility

S.aureus isolates were tested for their susceptibility to antimicrobial agents by Kirby-Bauer method on MHA (9) according to criteria recommended by Clinical and laboratory standards Institute (10).

Detection of IS 256

IS*256* element was detected in the twenty-two isolates of *S.aureus* first by isolation genomic DNA from early-exponential-phase cultures, then IS*256* was amplified by using specific primers; F: 5´-TAGAATTCG

GATAAAGTCCGTATAATTGTGT-3´ and R: 5´-TGTTCTAGCTAT ACAATGTTTTTACCATTTC-3´ (11). The conditions for amplification of IS*256* include the initial denaturation at 95°C for two minutes with 30 cycles consisting of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. per kb amplified.

3. Results and Discussion

Incidence of *S. aureus*

The *S.aureus* isolates were identified according to their biochemical characteristics as they were positive for acid production from lactose, N-acetyl-glucosamine, D-terhalose, D-mannitol, and D-maltose, while they were negative for acid production from amygdaline, arbutine, D-galactose, D-glucose, D-melibiose, D-raffinose, D-sorbitol, D-xylose, glycerol, L-arabinose and salicin. They were also urease negative, positive bacitracin resistance and growth in 6.5% NaCl.. The prevalence of these isolates among different clinical samples are in urinary tract infections, two out of 22 individuals examined (9.1%), wound 4(18.2%), sputum 3(13.6%), nasal vestibulum 4(18.2%), joint fluid 3(13.6%), operation soft tissue 3(13.6%), operation drainage 2(9.1%), and vagina 1(4.6%). *S.aureus* is now a common pathogen, which has been successfully established in the hospital environment.

Antibiotic susceptibility

The antibiotic susceptibility pattern of *S.aureus* isolates was studied. Results indicated in table (1) showed that multi-drug resistance was spread in these clinical isolates, it was found that most of the bacterial isolates (100%) were resistant to Penicillin and Methicillin, 77% were resistant to ampicillin, 54% to erythromycin, 45% to azithromycin, trimethoprim, and chloramphenicol,36% to tetracycline, 23% to ciprofloxacin, Lincomycin and Doxycycline, 18% to Gentamicin and 13% to Rifampin was resistance, 4% to vancomycin. Results also showed that the most antibiotic resistant isolates are the isolate no. S1 and 11 (78.5%), then S2 10(71%), then S5 and S8 9(64%), then S10 and S15 8 (57%), then S11 7(50%), then S4, S7, S14 and S21 6(43%), then S18 5(36%), then S12, S16, S17 and S20, 4(28%) then finally S6, S13, S20 and S22 were resistant to three (21%) of different antibiotics.

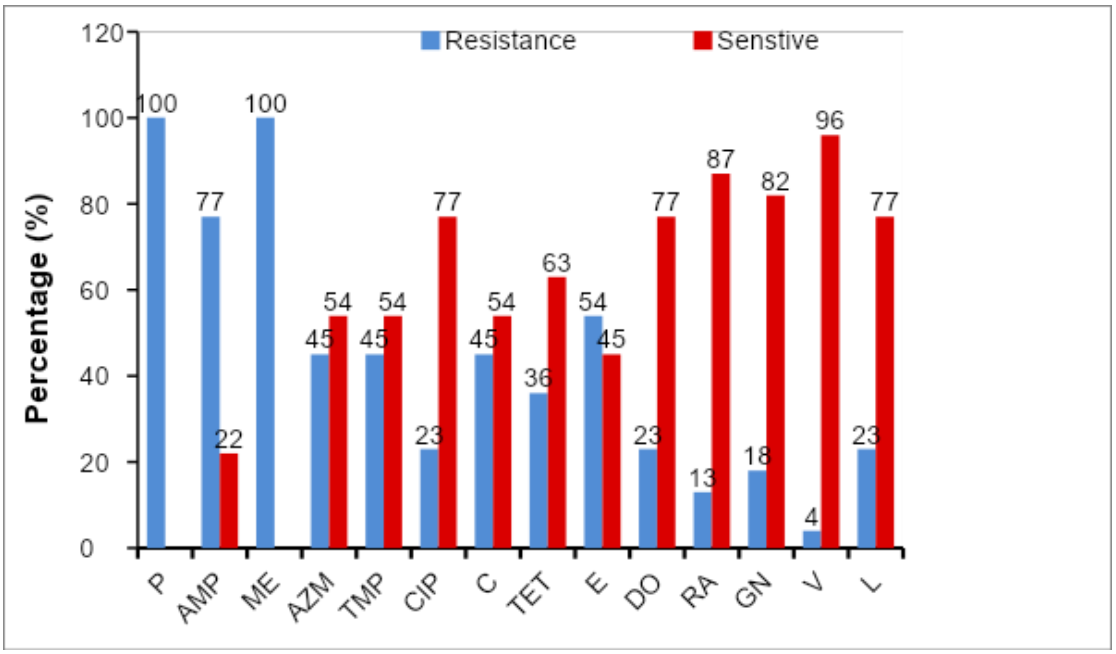

Figure 1: Antibiogram of *S.aureus* isolates and the susceptibility percentage against different antibiotics. P: penicillin G; AMP: Ampicillin; ME: Methicillin; AZM: Azethromycin; TMP: Trimethoprim; CIP: Ciprofloxacin ; C: Chloramphenicol; TET: Tetracycline; E:Erythromycin; Do: Doxycycline; RA: Rifampin; GN: gentamycin; V: vancomycin; L: Lincomycin

The development of antibiotics resistance by *S. aureus* has involved acquisition of determinants by horizontal gene transfer of mobile genetic elements (12). These determinants may have evolved in antibiotic producers to protect them from potentially inhibitory molecules, or in their competitors. Analysis of the soil resistome shows that bacteria that express resistance to antibiotics are widespread (13).

Identification of antibiotic resistance genes provides valuable information; however, Knowledge about their association with mobile genetic elements is crucial for the assessment of the risk for acquisition and dissemination of antimicrobial resistance. Transposable elements can be distributed on both chromosome and plasmids, and are able to interact by a recombination between elements and/or by transposition into other elements, forming all kinds of novel chimerical structures (14).

Detection of IS*256* in *S. aureus* clinical isolates

In this study, we wanted to investigate whether pathogenic *S. aureus* differ with respect to the presence of IS*256* in their genomes. This element occurs in multiple, independent copies in the genomes of staphylococci and enterococci (15, 16). Results showed that IS*256* was detected in the genome of 18 *S. aureus* clinical isolates (82%), while it was not detected in other four isolates (18%). On the other hand, results illustrated in figure (2) showed that IS*256* was

prevalent in all isolates (100%) from urinary tract infections, operation drainage tip, nasal cavity, wounds, and operation soft tissues, while the element was detected in two isolates (66.6%) from sputum, one isolate (33.3%) from joint fluid, and it was not detected in the vaginal isolate. However, the isolates differed with respect to the occurrence of IS*256* elements in their genomes. Specifically, the presence of IS*256* seems to be a feature of pathogenic *S. aureus* isolates. The presence of IS*256* might play a role in the flexibility of the genome of multiresistant *S. aureus* and could represent an advantage in the rapid adaptation of the bacterium to changing environmental conditions, and the underlying genetic mechanisms (17).

IS*256* element is present in multiple copies in the Staphylococcal genome, either flanking the Tn *4001* or independent of it. Inter –IS *256* PCR analysis is proposed as an efficient molecular typing assay for epidemiological studies of MARSA (19).

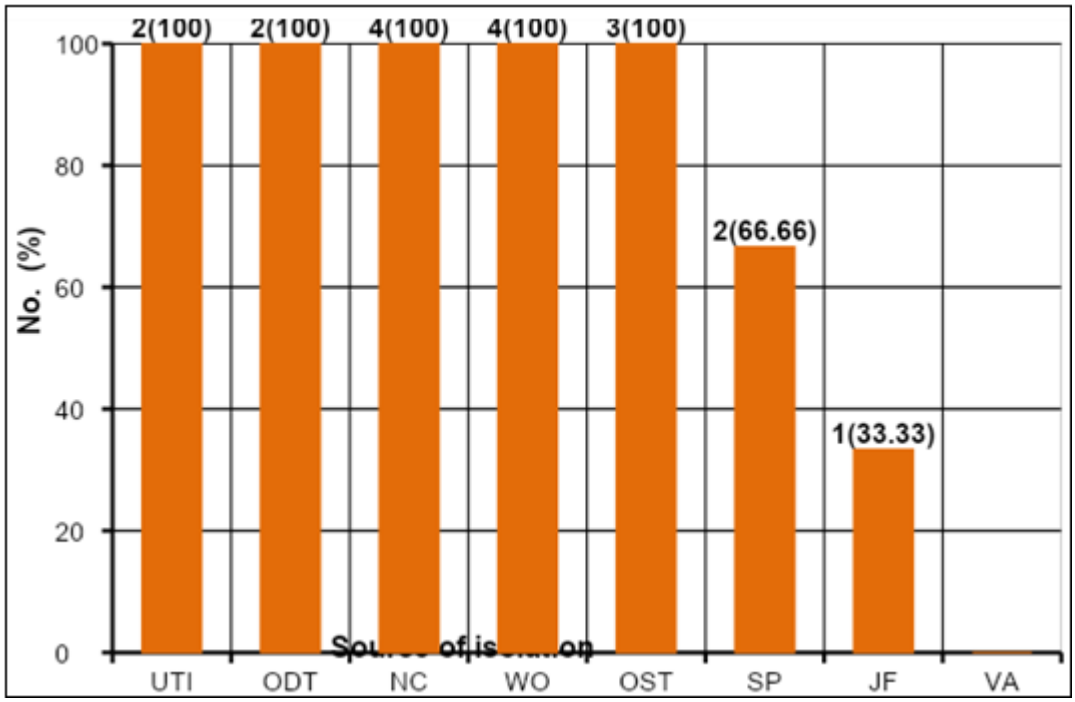

Figure 2: Distribution of IS*256* among *S. aureus* isolates from different clinical samples

On the other hand, results illustrated in figure (1) showed that 89.1% of the bacterial isolates were resistant to gentamycin, this may be because IS*256* is part of the composite transposon Tn*4001*, which mediates gentamicin resistance by the bifunctional aminoglycoside-modifying enzyme (17,18). In this study, IS*256* was detected in MRSA *S. aureus* isolates. This data confirmed previous results mentioned that multiple copies of IS*256* are also present in *S. aureus* strains that display intermediate resistance to vancomycin, the MRSA *S. aureus* are resistant to macrolides, rifampicin, quinolones, tetracycline, clindamycin, aminoglycosides, and chloramphenicol (19).

IS*256* is an insertion sequence wide spread in the genomes of multiresistant *Enterococci* and *staphylococci* (20). Therefore, the element, which is 1,324 bp in size, consists of a single open reading frame encoding a transposase protein flanked by noncoding regions harboring imperfect inverted repeats (17). IS*256* occurs in multiple free copies in its host genomes but is also known to form the ends of composite transposon Tn*4001* conferring aminoglycoside resistance. multiple genomic IS*256* copies may serve as crossover points for homologous recombination events and thereby play an important role in genome flexibility, adaptation, and evolution of staphylococcal and enterococcal genomes(22-23).

4. Conclusions

We found that IS*256* represents a specific element, which is more likely to occur in *S.aureus* clinical isolates from nosocomial infections. They were more frequently occurred in nasal cavity, urinary tract infections, wounds, operation drainage, and operation soft tissues than in sputum, joint fluid, and vagina respectively.

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