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USING SDS FOR PLASMID CURING TO STUDY THE ANTIBIOTIC RESISTANCE OF BACTERIA ISOLATED FROM ACUTE SUPPURATIVE OTITIS MEDIA SUBJECTS IN EGYPT

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ABSTRACT

The threat of antibiotic resistance is increasing all over the world, and treating bacterial infections has become more and more difficult. The aim of the present study was to determine the antibiotic sensitivity pattern of bacteria previously isolated from acute suppurative otitis media (ASOM) subjects, and find out whether their antibiotic resistance is a chromosomal or a plasmidic trait. An antibiotic sensitivity test was carried out for 78 isolates (66 Staphylococcus aureus isolates and 12 Streptococcus pneumoniae isolates) previously isolated from 76 ASOM subjects who visited Kasr El Ainini teaching hospital from February 2009 - August 2009. Out of 78 isolates, 69 (88.46 percent) were sensitive to Ciprofloxacin, followed by 52 (66.66 percent) sensitive to Cefotaxime, 47 (60.25 percent) sensitive to Amoxyclav, and 39 (50 percent) sensitive to Amoxicillin. Only 4 (5.12 percent) were sensitive to Ampicillin. Plasmid curing was then carried out followed by antibiotic sensitivity tests, and the results were compared with those obtained before plasmid curing. The results showed that the resistance of S.aureus to Amoxicillin, Amoxyclav, and Cefotaxime was plasmid mediated while its resistance to Ampicillin and Cirofloxacin was chromosome mediated. And the resistance of S. pneumoniae to Ampicillin and Amoxyclav was plasmid mediated, whereas its resistance to Amoxicillin, Cefotaxime, and Ciprofloxacin was chromosome mediated.

Keywords: Antibiotic resistance- Sodium Dodecyl Sulphate- Plasmid Curing- Chromosomal resistance- Plasmidic resistance- Acute suppurative otitis media- Otitis media.

INTRODUCTION

The rapid emergence of resistant bacteria is taking place globally, jeopardizing the efficacy of antibiotics, which have changed many pathways in medicine and saved millions of lives.[1-4] Accordingly, the management of (ASOM) is becoming complicated.[5] Studying the antibiotic sensitivity of the causative bacteria of ASOM is crucial for planning a general outline of treatment. In ASOM, antibiotic therapy includes amoxicillin. In patients who are not responding to ampicillin or amoxicillin, amoxicillin/ clavulanate potassium may be used. For those who are allergic to penicillin derivatives, therapy may include cefaclor, or co-trimoxazole. Ceftriaxone is effective against major pathogens but is expensive and is reserved for very sick infants. In the patients with recurring otitis media, antibiotics must be used with discretion to prevent development of resistant strains of bacteria.[6] Penicillin has been considered to be the treatment of choice for infections caused by *S.pneumoniae* for many years.

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Among antibiotics which are effective against *S.pneumoniae*, cefazolin has the lowest minimum inhibitory concentration (MIC). Vancomycin appears to be a suitable substitution for penicillin.[7] Most *S.aureus* strains are sensitive to a reasonable range of commonly used antibiotics, e.g. flucloxacillin (a penicillinase-resistant penicillin), erythromycin, and some of the cephalosporins. Gentamicin is also active against *S.aureus*. The last decade has seen the emergence of strains of S.aureus resistant to flucloxacillin and all cephalosporins. Some are also resistant to gentamicin, erythromycin, and chloramphenicol and in general are sensitive only to the glycopeptides antibiotics, vancomycin and teicoplanin, and these have to be given parenterally. These strains are known by the term methicillin-resistant *S.aureus* (MRSA). MRSA accounts for up to half of all *s.aureus* isolates in hospitals and is also emerging as a problem in the community, with the emergence of community-acquired MRSA.[8]

Non genetic resistance plays a less important role in development of antibiotic resistance. There are several non-genetic reasons for the failure of antibiotic to inhibit the growth of bacteria. Some bacteria can be walled off in certain conditions as in abscess cavity. This prevents antibiotics from penetrating effectively into bacteria. However, surgical drainage of the abscess makes bacteria susceptible again to antibiotics.[9] Under certain conditions, some bacteria which are killed normally by penicillin, can lose their cell wall and survive as protoplasts, and thus become insensitive to cell wall active antibiotics. But later, if these bacteria resynthesize their cell walls they return to being susceptible to these antibiotics.[10] Some non-replicating bacteria remain in their resting stage to the action of cell wall inhibitors such as penicillins and cephalosporins. However, when these bacteria begin to multiply, they become susceptible to antibiotics.[9]

Genetic basis of resistance plays the most important role in antibiotic resistance. Antibiotic resistance genes are present on both the bacterial chromosome and plasmids.[11] Chromosomal resistance occurs due to mutations which can be spontaneous or complex accumulated mutations.[12] Despite the fact that chromosome is the minimal genetic requirement for bacteria survival, some bacteria have non- essential pieces of DNA called plasmids. These minute circular extrachromosomal strands are found free or integrated into the chromosomes. They are not vital for bacteria growth and metabolism, but they impart protective traits such as antibiotic resistance and toxins production.[13] Many bacterial species developed resistance to various antibiotics as a result of plasmid exchange through transformation or conjugation.[14] The plasmid can be cured by increasing the incubation temperature or by treatment with chemicals such as mitomycin, ethidium bromide, acriflavine, or Sodium Dodecyl Sulfate (SDS).[15,16] The aim of this study is to determine the antibiotic sensitivity pattern of bacteria isolated from acute suppurative otitis media (ASOM) subjects, and find out whether their antibiotic resistance is a chromosomal or a plasmidic trait.

EXPERIMENTAL

Antimicrobial Susceptibility Test Pre- and Post- Plasmid Curing

Antibiotic susceptibilities were determined by Bauer and Kirby disk diffusion method. Isolates of *S.aureus* and *S. pneumoniae* were grown overnight in nutrient broths and cooked meat broths respectively. They were then swabbed onto Muller Hinton Agar (MHA) and Muller Hinton Agar (MHA) with 5% sheep blood plates respectively, and antibiotic discs were placed on the surface. The antibiotics tested were Ampicillin 10 µg, Amoxicillin 25 µg, Amoxyclav 30 µg, Cefotaxime 30 µg, and Ciprofloxacin 5 µg. All plates were then incubated at 37 °C for 24 hrs. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI).

Plasmid Curing

Plasmid curing was carried out according to the modified methods of Vyvyan et al., and Kai et al.[17,18]. Overnight cultures were used to inoculate broths containing serial dilutions of Sodium Dodecyl Sulfate (SDS). After Inoculation, the cultures were incubated at 37 °C for 24 hrs. The cultures in which no growth was observed were discarded, and sublethal concentrations were determined. Those were then incubated at 37 °C for 48 hours in order to confirm there was the growth of the tested organisms. Freshly prepared nutrient broths were again prepared and then inoculated with the plasmid-cured tested cultures and incubated at 37 °C for 24 hours. Then Antibiotic sensitivity was carried out again according to Bauer and Kirby disk diffusion method.

RESULTS AND DISCUSSION

Antibiotic Sensitivity Pattern of Isolates

Table 1 shows the sensitivity pattern of the *S.aureus* and *S. pneumoniae* isolates to the employed antibiotics: Ampicillin 10µg, Amoxicillin 25µg, Amoxyclav 30 µg, Cefotaxime 30µg, and Ciprofloxacin 5µg. Out of the 78 isolates, 69(88.46%) were sensitive to Ciprofloxacin, followed by 52(66.66%) sensitive to Cefotaxime; 47 (60.25%) were sensitive to Amoxyclav, followed by 39 (50%) sensitive to Amoxicillin. Only 4 (5.12%) were sensitive to Ampicillin.

Resistance Pattern of Isolates before and after Plasmid Curing

Table 2 shows the resistance pattern of the *S.aureus* and *S. pneumoniae* before and after curing to the employed antibiotics. After plasmid curing, *S.aureus* didn't show any sensitivity change to both Ampicillin and Ciprofloxacin, while there was 3.8 percent reduction in resistance to Amoxicillin, and 28.79 percent reduction in resistance to both Amoxyclav and Cefotaxime. *S. pneumoniae* showed 8.33% reduction in resistance to Ampicillin, 25% reduction in resistance to Amoxyclav, and did not show any reduction in resistance to Amoxicillin, Cefotaxime, and Ciprofloxacin. This suggests that the resistance of *S.aureus* to Amoxicillin, Amoxyclav, and Cefotaxime was plasmid-mediated while its resistance to Ampicillin and Cirofloxacin was chromosome mediated. And the resistance of *S. pneumoniae* to Ampicillin and Amoxyclav was plasmid mediated, whereas its resistance to Amoxicillin, Cefotaxime, and Ciprofloxacin was chromosome mediated.

Table-1: Incidence of antibiotic susceptibility of isolates

Microorganism	Total No. of isolates	Ampicillin 10µg	Amoxicillin 25µg	Amoxyclav 30 µg	Cefotaxime 30µg	Ciprofloxacin 5µg
		No. of sensitive isolates (%)				
S.aureus	66	3 (45.4)	35 (53.03)	40 (60.6)	42 (63.63)	58 (87.87)
S. pneumoniae	12	1 (8.33)	4 (33.33)	7 (58.33)	10 (83.33)	11 (91.66)
Total	78	4 (5.12)	39 (50)	47 (60.25)	52 966.66)	69 (88.46)

The study was carried out to evaluate the efficacy of the antibiotics prescribed at Kasr El Aini Teaching Hospital through carrying out antibiotic sensitivity tests, and find out whether the antibiotic resistance of the causative bacteria is a chromosomal or a plasmidic trait. Antibiotic sensitivity was carried out in the present study for 78 isolates by Kirby-Bauer disc diffusion method. Our results showed that 88.46 percent of the isolated microorganisms were sensitive to ciprofloxacin, followed by percent sensitive to Cefotaxime 63.63, 60.6 percent sensitive to Amoxyclav, 53.03 percent sensitive to Amoxicillin, and 4.54 percent sensitive to Ampicillin. The most effective antibiotics were Ciprofloxacin followed by Cefotaxime, and Amoxyclav, which showed higher activity against the isolates than Amoxicillin and Ampicillin.These results are similar to those obtained by Maithem A. Al-Hamdani and Intisar G. Hamad in Basrah, Iraq where *S. aureus* isolated from acute and chronic suppurative otitis media patients showed resistance to Ampicillin and Amoxicillin, and sensitivity to Ciprofloxacin.[19] In Erbil, Iraq, the antibiotic susceptibility testing carried out for *S. aureus* identified from burns, otitis media, wounds and urine infections by Abdulrahman et al. showed 94 percent resistance to Ampicillin, but all the isolates were sensitive to amoxicillin.[20] In another study, Akinjogunla et al. found that (66.7 percent) of *S.aureus* isolated from acute otitis media patients in Uyo, Nigeria were sensitive to ciprofloxacin.[21] On the contrary, *S. aureus* isolated from cases of otitis media in Benin, Nigeria by Orhue O. P. et al. were resistant to Cirprofloxacin.[11] Also, the results introduced by S.K.S. Ojo et al. showed that 57 percent of the staphylococci isolated from wounds and burns in Ekpan, Nigeria were resistant to Ciprofloxacin.[22] In a surveillance study in which organisms were isolated from specimens obtained from outpatients in 6 geographic regions of the United States, 94% of *S. pneumoniae* isolates were susceptible to amoxicillin and Amoxyclav.[23] In another study in Greece, all isolates of *S.pneumoniae* obtained from children with acute otitis media were found to be susceptible to cefotaxime.[24] Recently, *S.pneumoniae* isolated from acute otitis media patients in Taipei, Taiwan showed resistance rates of 66.3 percent and 20.2 percent to Amoxicillin and Cefotaxime, respectively.[25]

The difference in the pattern may be attributed to the difference in bacteria isolated and their various virulence factors.

After plasmid curing, our results showed that the resistance of *S.aureus* to Amoxicillin, Amoxyclav, and Cefotaxime was plasmid-mediated while its resistance to Ampicillin and Cirofloxacin was chromosome mediated. And the resistance of *S. pneumoniae* to Ampicillin and Amoxyclav was plasmid mediated, whereas its resistance to Amoxicillin, Cefotaxime, and Ciprofloxacin was chromosome mediated. Orhue O. P. et al. found that *S. aureus* isolated from cases of otitis media exhibited 100 percent plasmid resistance to Ciprofloxacin and 50 percent plasmid resistance to Amoxiclav.[11] S.K.S. Ojo et al. found that most of the resistance of staphylococci isolated from wounds and burns to various antibiotics including Ciprofloxacin was plasmid mediated.[22] In addition, the emergence and spread of plasmid-borne resistance genes which are expected to go through horizontal gene transfer have threatened many last-line antibiotic therapies, including quinolones such as Ciprofloxacin.[26]

Table-2: Resistance pattern of isolates before and after plasmid curing

Microorganism	Total No. of isolates	Ampicillin 10µg		Amoxicillin 25µg		Amoxyclav 30 µg		Cefotaxime 30µg		Ciprofloxacin 5µg	
		No. of Resistant isolates (%) before and after Curing									
		Normal	Cured	Normal	Cured	Normal	Cured	Normal	Cured	Normal	Cured
S.aureus	66	63 (95.45)	63 (95.45)	31 (20.46)	11 (16.66)	26 (39.39)	7 (10.6)	24 (36.36)	5 (7.57)	8 (12.12)	2 (3.03)
S. pneumoniae	12	11 (91.66)	10 (83.33)	8 (66.66)	8 (66.66)	5 (41.6)	3 (25.0)	2 (16.66)	2 (16.66)	1 (8.33)	1 (8.33)
Total	78	74 (94.87)	73 (93.58)	39 (50.0)	19 (24.35)	31 (39.74)	10 (12.82)	26 (33.33)	7 (8.97)	9 (11.53)	3 (3.84)

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