

Original Research Article

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Antibiotic Susceptibility of *Streptococcus pyogenes* Isolated from Otitis Media and Tonsillitis among Children Patients

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ABSTRACT

Keywords

Tonsillitis, Otitis media, *Strep. pyogenes*, Antibiotic susceptibility profile.

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Two hundred specimens (100 males and 100 females) were included, through (100) swabs of otitis media and (100) swabs of tonsillitis. These swabs had obtained from patients consulted to different hospitals of Baghdad city during the period from 10 March 2017 to 12 May 2017. There were 27(27%) positive isolates for *Strep. pyogenes*, which obtained from patients with age ranged from less than 6 years, who suffered from tonsillitis and ear infections. The phenotypic characterizations of twenty-seven isolates (13.5%) of *Strep. pyogenes*, which had obtained, showed characteristic colony morphology when cultured on different agar plates, microscopic appearance and biochemical tests. Twenty-seven (27) isolates of *Strep. pyogenes* were tested for their antibiotics susceptibility against eleven antibiotics by using Kirby-Bauer disc diffusion methods on Muller Hinton agar. Results of antibiotic susceptibility had showed different levels of sensitivity and resistance.

Introduction

Streptococcus pyogenes is Gram-positive, Non-motile, and non-spore forming spherical cocci with 0.5 to 1.0 μm , which can form long chains in liquid medium (1). *Streptococcus Pyogenes* causes several infections as pharyngitis, tonsillitis, cellulitis, necrotizing fasciitis, pneumonial bacteremia and ear infection, besides post infectious sequelae like acute rheumatic fever (6). Pathogenesis of *Streptococcus pyogenes* infections is multi factorial, as suggested by the number and wide array of virulence determinants possessed by cell-surface molecules which had reported as possible adhesions due to their ability to bind specific components of

Pathogenic bacterial strains that develop or acquire resistance to one or more first-line antibiotics pose numerous challenges to healthcare. These resistances are including increased patient morbidity and mortality, prolonged illness duration, and more expensive disease control measures (3, 4). Antibiotics resistance patterns were a serious and growing phenomenon in contemporary medicine and had emerged as one of the pre-eminent public health concerns of the 21st century. So that, there had a growth in the change and severity of *Streptococcus Pyogenes* contagions, including those that

were aggressive. However, *Streptococci* bacterium remnants vulnerable to penicillin, some strains had recognized to be resilient to macrolides, such as erythromycin, as well as tetracycline and clindamycin. This can cause *Streptococcus spp.* isolates to be exposed to ineffective antibiotics treatments and develop further resistance (5). Moreover, the rapidly growing problems of antibiotic resistant *Streptococcus pyogenes* have existed and increased according to severity of pathogenic agents, immunity of individuals, geographical locations and investigators (7,8). Furthermore, several genetic elements involved in antibiotic resistance had horizontally transferred among different Lancefield groups and within species (9,10).

Materials and Methods

Patients

Two hundred patients (100 males and 100 females) with age less than 6 years, who suffered from tonsillitis and ear infection, that presenting to five hospitals of Baghdad city during the period from 10 March 2017 to 12 May 2017.

Specimen's collection

Tonsillitis and otitis media specimens were collected using cotton swabs. These swabs had directly inoculated onto the plates of transport medium, then incubated overnight at 37°C (14). The history for each case had recorded in a questionnaire at case sheets.

Identification of *Streptococcus pyogenes*

Culturing of throat and ear specimens

Each swab was immediately taken by transport medium and then inoculated on blood agar plates and 5% sheep blood agar plates and incubated at (5-10%) CO₂ to prove

the facultative anaerobic-enriched atmosphere for 24 hours at 37°C (14). In addition, different media had used for laboratory isolation and identification.

Colony morphology and microscopic

A single colony had taken from each primary positive culture and its identification depended on the morphology properties (colony, size, shape, color and natural of pigments, translucency, edge, elevation and texture).

Suspected colonies had selected and investigated by gram stain to observe as specific color reaction, shape aggregation and specific intracellular compounds after staining the bacteria by gram stains, tests had done to reach the final diagnosis (15).

Statistical analysis

Statistical program had performed by SPSS (V-15). Descriptive statistics had included tables that create (number, percentage, mean and standard error (16).

Results and Discussion

Isolation and identification

Macroscopic and microscopic characterizations

From two hundred samples had obtained from tonsillitis and otitis media infections patients presenting to five hospitals in Baghdad city. The phenotypic characterization of twenty-seven isolates (27%) of *Streptococcus pyogenes*, which had obtained showed characteristics colony morphology when cultured on blood agar plates, so its appear as white to grey colonies (1-2) mm in diameter (15). While macroscopic features of *Streptococcus pyogenes* isolates reported G

+ve cocci chains or pairs non-motile bacterium and non-spore forming cells (15).

Biochemical tests

All 27 *Streptococcus pyogenes* isolates were positive for oxidase, catalase, Simmon's citrate utilization, in dole negative and negative MR-VP tests. It did not ferment carbohydrates, but many isolates oxidize glucose. All these results had confirmed by Mini API 20 STREP system and VITEK 2 system *Streptococcus pyogenes* (16).

Antibiotic susceptibility

Antibiotic Susceptibility of *Streptococcus Pyogenes* isolates had showed different levels of sensitivity and resistance as follow:

Benzyl penicillin

Streptococcus pyogenes isolates had showed highly sensitivity 25(92.6%) and 2 (7.4%) resistant isolates, with highly significant differences ($P < 0.01$). These findings agree with Chaurasia *et al.*, (2014) (18) who mentioned that the sensitivity of *Streptococcus pyogenes* isolates to benzyl Penicillin was 80.2(78.3%). While Muluye *et al.*, (2014) (19) reported that *Streptococcus pyogenes* isolates were resistance to the benzyl Penicillin 628 (59.8%), and these results disagree with current results (Table 1).

Ampicillin

In current, study *Streptococcus pyogenes* isolates were sensitive to Ampicillin antibiotic 24(88.88%), resist 3(11.12%) and observed highly significant differences ($P < 0.01$). (Table 1) Prajapati *et al.*, (2014) (20) reported that the *Streptococcus pyogenes* isolates were resistance to Ampicillin 51 (7.8%), through that these results nearly with current study.

Sanjeeb *et al.*, (2014) (21) stated that the *Streptococcus pyogenes* isolates were resistance to Ampicillin 123 (38.1%) and these results disagree with current study.

Penicillin

Results of susceptibility was that *Streptococcus pyogenes* isolates were sensitive to Penicillin 16(59.26%), resistance 11(40.74%) and there were non-significant differences ($P > 0.05$).

Curtin-Wirt *et al.*, (2003) (22) reported that isolates of *Streptococcus pyogenes* were sensitive to Penicillin 276(64%) these results agree to current study. So results of current study were disagreed with Al-Saimary (2011) (11) who reported that isolates of *Streptococcus pyogenes* were resist to Penicillin 24(100%).

Fig.1 Antibiotic susceptibility pattern of *Streptococcus pyogenes* isolates

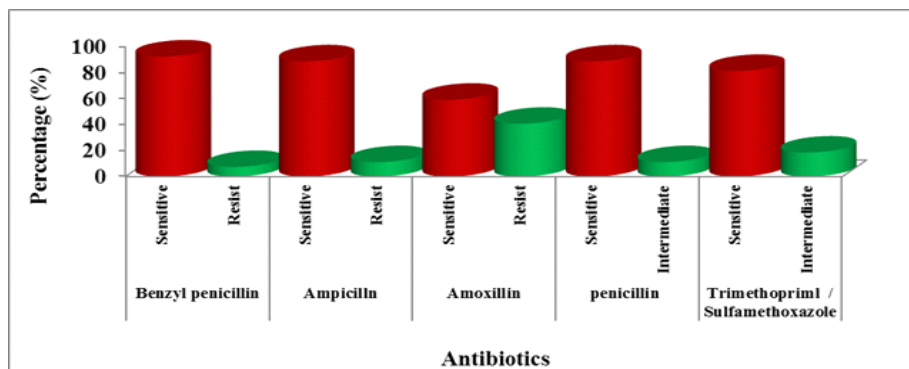


Fig.2 Antibiotic sensitivity pattern of *Streptococcus pyogenes* isolates

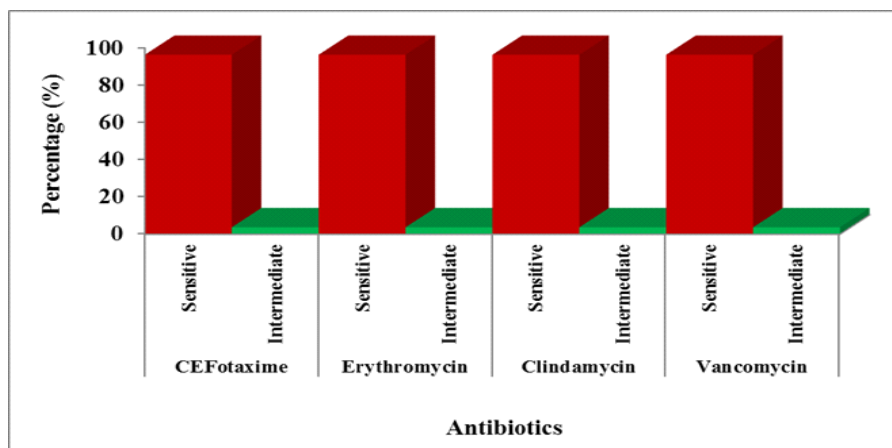


Table.1 Antibiotic Susceptibility Pattern for *Streptococcus pyogenes*

Antibiotic		No.	%	Z-test (P-value)
Benzyl penicillin	Sensitive	25	92.6%	P=0.00 HS (P<0.01)
	Resist	2	7.4%	
Ampicillin	Sensitive	24	88.88%	P=0.00 HS (P<0.01)
	Resist	3	11.12%	
Penicillin	Sensitive	16	59.26%	P=0.442 NS (P>0.05)
	Resist	11	40.74%	
Trimethoprim /Sulfamethoxazole	Sensitive	22	81.48%	P=0.002HS (P<0.01)
	Intermediate	5	18.52%	
Cefotaxim	Sensitive	26	96.3%	P=0.00 HS (P<0.01)
	Intermediate	1	3.7%	
Erythromycin	Sensitive	26	96.3%	P=0.00 HS (P<0.01)
	Intermediate	1	3.7%	
Clindamycin	Sensitive	26	96.3%	P=0.00 HS (P<0.01)
	Intermediate	1	3.7%	
Vancomycin	Sensitive	26	96.3%	P=0.00HS (P<0.01)
	Intermediate	1	3.7%	

Trimethoprim/sulfamethoxazole or co-trimoxazole

The results of study were reported that *Strep pyogenes* isolates showed sensitive 22(81.48%) and intermediate 5(18.52) with highly significant differences (P<0.01). Agrawal *et al.*, (2014) (12) showed

Streptococcus pyogenes isolates resistant to co-trimoxazole 100 (66.67%), and these findings differ from results of present study. While Bergmann, (2014) (13) explained that *Streptococcus pyogenes* isolates showed intermediate resistance to trimethoprim, which were 69(1.5%) and these results similar current study (Figure 1).

Cefotaxim

The results of present study showed a highly significant differences ($P<0.01$). And the sensitivity to cefotaxim were 26(96.3%), intermediate 1(3.7%). These results disagree with Ramadan *et al.*, (2010) (23) who reported that the *Streptococcus pyogenes* isolates resistant to Cefotaxim 42(38%) (Figure 2).

Erythromycin

In this study, *Streptococcus pyogenes* isolates were sensitive to Erythromycin 26(96.3%), intermediate 1(3.7%) with highly significant differences ($P<0.01$). These results agree with Prajapati *et al.*, (2012) (20) who showed that the *Streptococcus pyogenes* isolates sensitive to 47 (92.2%). In addition, these results agreed with results of Ray *et al.*, (2004) (24) reported that *Streptococcus pyogenes* isolates sensitive to Erythromycin which was 103 (89.6%).

Clindamycin

Clindamycin susceptibility results showed that there were 26 isolates of *Streptococcus pyogenes* appeared 96.3% sensitivity and 1 (3.7%) intermediate with highly significant differences ($P<0.01$). These findings approach with Ray *et al.*, (2004) (24) who showed that the *Streptococcus pyogenes* isolates were sensitive to Clindamycin with 115 (100%). While Glauber *et al.*, (2014) (25) reported that resistance of same isolates to Clindamycin were 91 (15.4%). Catalina, *et al.*, (2011) (26) reported that the *Streptococcus pyogenes* isolates sensitive to Clindamycin were (85%) and these findings disagree with results of present study.

Vancomycin

Results of *Streptococcus pyogenes* isolates sensitivity was showed 26 (96.3%) and

intermediate was 1 (3.7) with non-significant differences ($P<0.01$). These obtained results agree with Agrawal *et al.*, (2014) (12) who presented that 6(100%) of *Streptococcus pyogenes* isolates were sensitive to Vancomycin. While Capoor *et al.*, (2006) (27) disagree with current study when reported that 10(29.4%) of *Streptococcus pyogenes* isolates sensitive to Vancomycin.

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