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Frequency and antibiogram of *Streptococcus* spp isolated from different specimens: Three years of study

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ABSTRACT

Streptococcus is involved in mild and severe infections with a high level of antimicrobial resistance. This study aimed to investigate the prevalence and multi-drug resistance of *Streptococcus* spp isolates for three years (2016, 2017, and 2018). 1648 participants (246 males and 1402 females) were enrolled. Specimens were collected and transferred to the laboratory. All isolates were examined and identified according to standard methods. Susceptibility to antibiotics was evaluated utilizing the method of disk diffusion. Overall, *Streptococcus* spp were confirmed in 124 (7.52%) patients. The UTIs rate was significantly higher (76.6%) compared with other infections. The infected females were considerably higher than the infected males, 64.5% and 12.1% respectively. Higher percentages of *Streptococcus* spp were observed in 2017 (41.3%). The frequency of *Streptococcus* was higher in January compared with other months of the year. *Streptococcus* spp and *S. pyogenes* dominated over these months. The highest frequency of *Streptococcus* spp was found in age groups 16-20 and 21-25 (22/18.49%, and 26/21.85% respectively). Multi-drug resistance was found among 36 (81%) of *Streptococcus pyogenes*, 50% (5/10) of *Streptococcus viridans* and 75% of *Streptococcus faecalis*. The overall *Streptococcus* spp showed 90 (72.6%) multi-drug resistance. High resistance was recorded to different antibiotics, Ceftazidime (96.6%), Oxacillin (96.7%) and Cefixime (86.9%). The incidence of *Streptococcus* spp was high during the three years of study with high resistance to the most available antibiotics. Susceptibility testing should be carried out and the empirical antibiotic treatment should be altered accordingly.

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Introduction

Various species of the genus *Streptococcus* are opportunistic pathogens and are involved in numerous in humans and animals bacterial infections (1). The infection of these bacteria could be transmitted through the genito-urinary tract, infected animals (2). Among all Streptococcus spp, *S. pyrogens* are presenting a crucial problem in poor counties, causing many deaths annually (3), and it caused fatal post-delivery infections (4). *S.* pyogenes is identified as group A Streptococcus (GAS) infected humans with mild infections, including impetigo, rheumatic fever, and pharyngitis, and severe diseases, including streptococcal toxic shock syndrome and necrotizing fasciitis and urinary tract infections (5). *Streptococcus* spp have been reported a high level of anti-bacterial resistance to most anti-bacterial categories (6). The impact of multidrug-resist bacteria has become a global health concern socially and clinically; the resistance could be natural or acquired (7). To overcome anti-bacterial drugs, the resistance could be developed to increase the bacterial ability to survive and compete for the anti-bacterial activity even in recent generations of used anti-bacterial drugs (8). This study aimed to investigate the prevalence and multi-drug resistance of isolated gram-positive bacteria, *Streptococcus* spp, among admitted patients of Zakho hospital for three years (2016, 2017, and 2018).

Materials and Methods

Study design and setting

The cross-sectional study was conducted throughout three indicated years of study (2016, 2017, and 2018) at Zakho emergency hospital, Kurdistan region, Iraq. Overall, 1648 participants (246 male and 1402 females) were recruited in this study. Different specimens, including urine, sputum, and wound swabs were collected from admitted patients, with different clinical manifestations, throughout the stated period of study.

Specimen collection, identification, and antibiotic sensitivity test

The collected specimens were collected and immediately sent to the diagnostic microbiology laboratory and streaked directly on blood agar, chocolate agar and McConkey agar, which were incubated aerobically at 37°C for 24 hours. For purification and identification, isolates were examined for their Gram stain reaction and biochemical characteristics according to well-known established bacteriological methods (9). Biochemical identification of bacterial species was performed by standard procedures and international guidelines (10). Susceptibility to a range of antibiotics (Table 1) that were available and routinely used in the hospital, was evaluated utilizing the disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) 2015 (11).

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Table 1. The distribution of *Streptococcus* spp specimens among total specimens from referral patients to the Zakho hospital laboratory throughout three years (2016, 2017, and 2018).

	Total admitted patients		Patients with Streptococcus	
	Males	**Females**	**Males (%)**	**Females (%)**
Urine specimen	191	1372	15 (7.85)	80 (5.83)
Swab specimen	17	7	5 (29.41)	1 (14.29)
Sputum specimen	38	23	12 (31.58)	11(47.83)
Total	246	1402	32 (13)	92 (6.5)
Isolated bacteria	Urine	Swab	Sputum	Total
Streptococcus pyogenes	27	4	13	44
Streptococcus viridans	9	0	1	10
Streptococcus faecalis	3	0	1	4
Streptococcus epidermidis	1	0	0	1
Streptococcus spp.	54	2	8	64
Streptococcus pneumoniae	1	0	0	1
Total	95	6	23	124

Ethics Statement

The patients recruited were notified and the permission for the research design and methodology was approved by the scientific committee of the department of biology, Duhok University/ College of Science, Kurdistan, Iraq.

Data Analysis

SPSS 16 software was used for the statistical analysis of all data. The differences were considered significant at *P* value <0.05.

Results

The *Streptococcus* spp were isolated from different specimens, including urine, sputum, and wound swabs from admitted patients to Zakho hospital, Kurdistan region, Iraq, throughout three indicated years of study (2016, 2017, and 2018). Overall, 1648 participants (246 males and 1402 females) through the stated period of study, *Streptococcus* spp, were confirmed in 124 (7.52%) patients (Table 1). The UTIs rate was significantly (*P* value < 0.001) higher compared with other infections with 95 (76.6%) Streptococcus spp confirmed patients. Amongst the 124 isolated *Streptococcus* spp, the infected females were considerably higher (*P* value is < 0.001) than infected mals (80; 64.5% and 15; 12.1% respectively). However, the rate of other infections was higher in males compared with females (17; 13.7% and 12; 9.7% respectively) (Fig. 1B). The higher frequency/ percentages of identified *Streptococcus* spp were observed in 2017 (51; 41.3%) compared with 2016 (37; 29.84%) and 2018 (36; 29.03%) (Fig. 1A).

The incidence of *Streptococcus* spp (the genus *Streptococcus* isolates that species not identified) was higher in frequency followed by *Streptococcus pyogenes* isolates (64 and 44 isolates respectively) and the higher frequency was isolated from urine and sputum, whereas the lower frequency was isolated from wound swab spacemen (Table 1).

The frequency of patient's specimens identified with *Streptococcus* was higher in January, February, May, and June compared with other months of the year (Fig. 2D). And *Streptococcus* spp and *S. pyogenes* were dominated over these months. A relatively similar pattern in each year separately (Fig. 2A, B, and C). Less frequency was observed in March, July, August, and September throughout three years of study. Furthermore, the high frequency was followed in December 2017 compared with the same time in 2016 and 2018.

The frequency of confirmed *Streptococcus* infection was also studied according to the patient's age. The increasing pattern showed to be started from the age group five years or less to reach the highest frequency at the age group 16-20 (22; 18.49%) and 21-25 years (26; 21.85%) and decreased dramatically to be at the lowest frequency

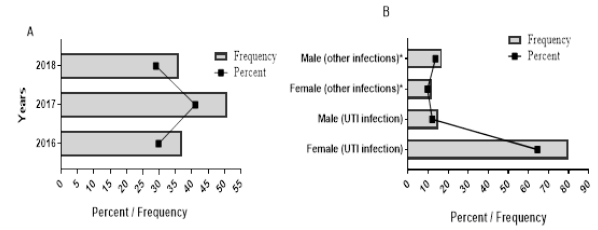

Figure 1. The distribution of *Streptococcus* confirmed infections. A: The Streptococcus confirmed cases frequency distributed according to the year of study. B: The gender base distribution among admitted patients with other bacterial infections, the infected patients to Zakho hospital.

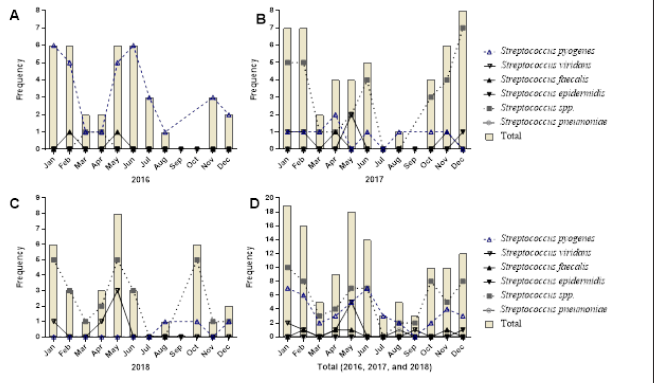

Figure 2. The distribution of frequency of *Streptococcus* confirmed infections in both genders according to the months of the year throughout three indicated years of study.

at age group 51-55 years (1; 0.84%). All three years of the study showed relatively the same pattern of infection according to the patients age group. The prevalence of the disease was shown to be increased from the age of five years and younger to reach the highest percentage of infection between 20 to 39 years old (98 patients, 47%), while the frequency of *E. coli* infection decreased dramatically from patients aged 40 years and older to reach the lowest incidence in elderly patients70-74 years (2, 1%) (Fig. 3).

The confirmed *Streptococcus* spp isolates were tested for antibacterial drug resistance reliant on the obtainability of these analysed anti-bacterial drugs used in the hospital diagnostic laboratory (at the time of the test). Moreover, all results (resistance, sensitive and moderate sensitivity against antimicrobial drugs) were included in the analysed data. The antibiotic sensitivity using disk diffusion test was routinely applied to all referred cases to identify the antibiotic sensitivity to available anti-bacterial treatments. As described in (Table 2), the investigated different species of *Streptococcus* showed a different level of resistance value to the analysed anti-bacterial treatments.

The results showed that about 36 (81%) of *Streptococcus pyogenes* were considered as multi-drug resistant (the resistance was shown to at least one of three or more antibiotic categories (12). Five isolates from 10 identified *Streptococcus viridans* were conceded as multi-drug resistant. While three isolated *Streptococcus faecalis* from four isolated bacteria (about 75%) were considered multi-drug resistance bacteria. In addition, the *Streptococcus* spp revealed 68.7% (44/64) of the isolates were multi-drug resistant. Both isolated *Streptococcus epidermidis* and *Streptococcus pneumonia* were considered multi-drug resistant. Collectively, the overall *Streptococcus* spp showed 90 (72.6%) were conceded as multi-drug resistance bacteria (Table 2). The bacterial isolates were considered multi-drug resistant after reporting resistance in at least one of three or more anti-bacterial categories (12).

Streptococcus spp showed high percentages of anti-bacterial resistance against most members of beta-lactamase antibiotics, about 86.6% of used beta-lactam antibiotics showed 50% or more antibiotic resistance against isolated *Streptococcus*. In addition, about 80 – 90% or more of analysed bacteria were resistant to the used beta-lactamase antibiotics, including Ceftazidime (96.6%), Oxacillin (96.7%), Cefixime (86.9%), Cloxacillin (83.3%), Cloxacillin (79.5%), and Ceftriaxone (79.2%). The analysed *Streptococcus* presented more than 70 % of resistance to erythromycin (macrolide antibiotics), Nalidixic acid (Fluoroquinolone) and Trimethoprim-Sulfamethoxazole, (Table 3). The isolated *Streptococcus* also showed a variable resistance activity against other antibiotics used in this study (Table 3). The high sensitivity of analysed *Streptococcus* was observed to both beta-lactamase antibiotics imipenem and meropenem (93.2 and 90.4% respectively).

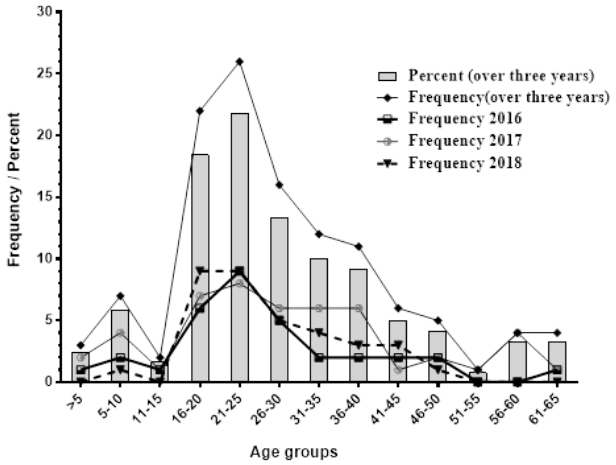

Figure 3. The distribution of *Streptococcus* confirmed infections in both genders according to the age groups throughout three indicated years of study.

Table 2. The incidence of multi-drug resistance of *Streptococcus* spp isolated from urine, sputum, and swab spacemen.

The incidence and percentages (%) of multi-drug resistance						
Infected bacteria	**Non-MDR (resistance to 2 or less anti-bacterial categories)**	**MDR for three anti-bacterial categories**	**MDR for four anti-bacterial categories**	**MDR for five anti-bacterial categories**	**Resistance to six or more anti-bacterial categories**	**Total number of isolates**
Streptococcus pyogenes	8 (18.2)	11 (25) * 36 (81.8)	10 (22.7)	11 (25)	4 (9.1)	44
Streptococcus viridans	5 (50)	3 (30) * 5 (50)	2 (20)	0 (0)	0 (0)	10
Streptococcus faecalis	1 (25)	1 (25) * 3 (75)	1 (25)	1 (25)	0 (0)	4
Streptococcus epidermidis	0 (0)	1 (100) * 1 (100)	0 (0)	0 (0)	0 (0)	1
Streptococcus spp.	20 (31.3)	29 (45.3) * 44 (68.7)	9 (14)	3 (4.7)	3 (4.7)	64
Streptococcus pneumoniae	0 (0)	1 (100) * 1 (100)	0 (0)	0 (0)	0 (0)	1
Overall identified Streptococcus	34 (27.4)	46 (37.1)	22 (17.7)	15 (12.1)	7 (5.7)	124

Table 3. Antimicrobial sensitivity test results for isolated *Streptococcus* spp from urine, sputum, and swab specimens separated according to the antibiotic categories.

Anti-bacterial Classes	Analyzed antibiotics	Concentration/disk (µg)	Antibiotic generations	Whole analysed specimens	S* n (%)	R* n (%)	MS* n (%)
B-lactamase	Ampicillin	10	3rd	68	14 (20.5)	42 (61.8)	12 (17.6)
	Amoxicillin	10	3rd	40	7 (17.5)	28 (70)	5 (12.5)
	Amoxicillin Clavulanic acid	20/10	4th	109	28 (25.7)	64 (58.7)	17 (15.6)
	Ampicillin/ Cloxacillin	10/10	4th	39	5 (12.8)	31 (79.5)	3 (7.7)
	Cefixime (CFM)	5	3rd	84	10 (11.9)	73 (86.9)	1 (1.2)
	Cefotaxime (CTX)	30	3rd	101	29 (28.7)	61 (60.4)	11 (10.9)
	Ceftazidime	30	3rd	29	1 (3.4)	28 (96.6)	0(0)
	Ceftriaxone (CRO)	30	3rd	77	11 (14.3)	61 (79.2)	5 (6.5)
	Cephalexin	30	2nd	20	3 (15)	14 (70)	3 (15)
	Cephalothin (KF)	30	1st	50	21 (42)	21 (42)	8 (16)
	Cloxacillin	10	2nd	36	3 (8.3)	30 (83.3)	3 (8.3)
	Penicillin	10	1st	61	18 (29.5)	37 (60.7)	6 (9.8)
	Imipenem (IMP)	10	NCG*	44	41 (93.2)	2 (4.5)	1 (2.3)
	Meropenem (MEM)	10	NCG	52	47 (90.4)	3 (5.8)	2 (3.8)
	Oxacillin	1	2nd	30	1 (3.3)	29 (96.7)	0 (0)
Tetracyclines	Doxycycline (DOX)	30	2nd	49	15(30.6)	21 (42.9)	13 (26.5)
	Tetracycline (TE)	30	NCG	45	17 (37.8)	21 (46.7)	7 (15.6)
Nitrofurans	Nitrofurantoin (NIT)	300	NCG	26	7 (26.9)	13 (50)	6 (23.1)
Fluoroquinolone	Ciprofloxacin (CIP)	5	2nd	81	33 (40.7)	34 (42)	14(17.3)
	Nalidixic acid (NA)	30	1st	38	3 (7.9)	32 (84.2)	3 (7.9)
	Ofloxacin	5	2nd	59	41 (69.5)	11 (18.6)	7 (11.9)
Aminoglycosides	Amikacin (AK)	30	NCG	104	58 (55.8)	29 (27.9)	17 (16.3)
	Gentamicin (CN	10	NCG	106	47 (44.3)	39 (36.8)	20 (18.9)
Rifamycins	Rifampicin	30	NCG	67	42 (62.7)	15 (22.4)	10 (14.9)
Macrolide	Azithromycin (AZM)	15	2nd	88	58 (55.8)	29 (27.9)	17 (16.3)
	Clindamycin (DA)	2	NCG	23	5 (21.7)	16 (69.6)	2 (8.7)
	Erythromycin	15	NCG	69	7 (10.1)	54 (78.3)	8 (11.6)
Others	Trimethoprim-Sulfamethoxazole (SXT)	1.25/23.75	NCG	36	7 (19.4)	27 (75)	2 (5.6)

S: Sensitive, R: Resistance, NCG: Non-Classified Generation. MS: Moderate sensitivity.

Discussion

Streptococcus spp are associated with a variety of diseases that infect humans and animals worldwide (13), in particular with raising the multi-drug resistance against the anti-bacterial treatment (7). This ability allows bacteria to overcome even the recent generations of anti-bacterial therapies. And this becomes a clinical and socioeconomic global dilemma (14). UTI infections, pharyngitis, and skin infections are among many illnesses caused by *Streptococcus* spp (5). These bacteria have the anti-bacterial resistance ability to various anti-bacterial treatments (6). The current study found that the most dominant theme of Streptococcus spp infection was found in the Urinary tract (Table 1, Fig. 1), and it was significantly higher in females. Every year, 150 million diseases of humans are reportedly suffering from UTIs (15). About 7.85% of hospital-admitted males and 5.83 of hospital-admitted females from total suspected UTIs were confirmed infected with *Streptococcus* over three years of study (2016, 2017, and 2018) (Table 1). It has been reported that 7.5% of UTI infections are caused by *Streptococcus spp* in children (16). Different aspects are associated with the distribution and spreading of UTIs, including age, gender, marriage status, hospitalization, socioeconomic, behaviors, genitourinary abnormalities, demographic data, clinical features, practices, and seasonal variation (17). The high percentages of UTIs infections were found in females compared with males. This result was in line with several researchers findings (18, 19). It exposed that about 50% of females have experienced a UTI at least once throughout their lifespan (20). It is thought that a high risk of women for UTI than men due to their physiological and anatomical characteristics (21). For instance, the closeness between the urethra/anus and the genital tract in females is one possible reason that could enhance the auto-transmission of the bacteria and increase the rate of UTIs in females (22).

In a study that collected data over 32 years (1980-2011), they found that the temperature in Iraq is highly varied from season to season, is averaged from 48^{o}C in July and August to below zero through January (23). It was indicated that a high percentage of Streptococcus infection was in January, February, May, and June, over three years of study (2016, 2017, and 2018) (Fig. 2D). And the high frequency of *Streptococcus* spp infections was observed in December 2017 (Fig. 2B). This high rate of infections through these three years, especially in 2017, could be because the high number of internally displaced persons (IDPs) from other Iraqi cities toward Zakho city when ISIS attacked many cities in Iraq in 2014. This large number of IDPs put pressure on the healthcare system and its institutions in the local area. By the end of 2015, around 200,000 internally displaced persons (IDPs) were living in camps sheltering in the Duhok governorate. Increasing the incidence of UTIs infections in winter months agreed with other studies (24), which found that most UTI-causative bacteria are seasonally distributed (25-27).

The incidence of *Streptococcus* spp infections was diverse according to the age groups (Fig. 3). The higher frequency of Streptococcus infections was at ages 16-30 years old, and the rate dramatically declined toward older generations, reaching the lowers frequency at age 51-55 years old. A research group (24) found that more than 50% of UTI infections were at ages 25-30 years old. It could be possible that the reason for increasing the frequency of Streptococcus infection in this age is that at this age is more interactive with society in terms of work, unhygienic food and water consumption outside the home or at the workplace or with friends or colleagues besides increasing the rate of social gathering (26, 28). In addition to the sexual activities and hormonal changes that promote the alteration of normal vaginal flora of females that might raise the risk of UTI infections (29).

In recent times, antibiotic has no longer been effective against pathogenic bacteria with emerging and developing multi-drug resistance bacterial isolates (30-32). In the current study, the multi-drug resistance streptococcus spp were observed. It has been indicated that the susceptibility to the antibiotics was variable and high resistance was observed against Ceftazidime, Oxacillin, Cefixime, Cloxacillin, and Cloxacillin. In addition to the resistance that showed against erythromycin (macrolide antibiotics), Nalidixic acid (Fluoroquinolone) and Trimethoprim-Sulfamethoxazole. It has been indicated that rifampicin and clindamycin were effective antibiotics against *Strep pyogenes* (4).

In this study, high sensitivity of analysed Streptococcus was observed to both beta-lactamase antibiotics imipenem and meropenem (93.2 and 90.4% respectively). These results come in line with other findings. *Streptococcus* spp was among other isolates from the human vagina and UTI infection which showed resistance to tetracycline (2, 33).

Antibiotic resistance can naturally occur independently of previous antibiotic exposure, or the resistance acquired due to chromosomal point mutations or by the acquisition of mobile resistance genes (34, 35). Previous studies from our region showed high levels of antibiotic resistance (36-39).

The rise of antibiotic-resistant streptococci has been associated with diverse mechanisms, such as efflux pumps and modifications of the antimicrobial target. Among streptococci, antibiotic resistance emerges from previously sensitive populations as a result of horizontal gene transfer or chromosomal point mutations due to excessive use of antimicrobials. Streptococci strains are also recognized as biofilm producers (40). The improper prescribing and the increased consumption of antimicrobials have led to selective pressure that triggers drug resistance in exposed bacteria and, consequently, in the persistence of antibiotic resistance genes in *Streptococcus* spp populations.

In this study, A high and steady frequency of *Streptococcus* spp was observed during the three years of study (2016, 2017, 2018). It has been indicated that the resistance of *Streptococcus* spp to the most available antibiotics was high in Zakho city. Susceptibility testing should be carried out on all clinical isolates, and the empirical antibiotic treatment should be altered accordingly.

Conflict of interest

The authors declare that there is no conflict of interest.

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