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ORIGINAL ARTICLE

Virulence factors and antibiotic resistance properties of Streptococcus species isolated from hospital cockroaches

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Abstract

Hospital cockroaches are probable sources of pathogenic bacteria. The present investigation was performed to assess the antibiotic resistance properties and distribution of virulence factors in the Streptococcus *spp*. isolated from hospital cockroaches. Six hundred and sixty cockroach samples were collected. Cockroaches were washed with normal saline, and the achieved saline was used for bacterial culture. Isolated Streptococcus spp. were subjected to disk diffusion. The distribution of virulence factors and antibiotic resistance genes were assessed using a polymerase chain reaction. The prevalence of *S. pyogenes, S. agalactiae,* and *S. pneumonia* amongst examined samples was 4.82%, 1.66%, and 6.96%, respectively. *Cfb* (53.93%), *cyl* (52.8%), *scaa* (51.68%) and *glna* (50.56%) were the most commonly detected virulence factors. *Pbp2b* (71.91%), *pbp2x* (58.42%), *mefA* (46.06%), *ermB* (46.06%) and *tetM* (46.06%) were the most commonly detected antibiotic resistance genes. Streptococcal spp. harbored the highest prevalence of resistance against tetracycline (80.89%), trimethoprim (65.16%), and penicillin (56.17%). To the best of our knowledge, this is the first prevalence report of virulence factors and antibiotic resistance genes in the Streptococcal spp. isolated from American, German, and oriental hospital cockroaches in Iran. Our findings indicated a certain role for cockroaches in nosocomial pathogens transmission in the hospital environment.

Keywords Streptococcus species · Virulence factors · Antibiotic resistance · Cockroaches · Hospital

Abbreviations

S. pneumoniae	*Streptococcus pneumonia*
S. pyogenes	*Streptococcus pyogenes*
S. agalactiae	*Streptococcus agalactiae*
bac	Encode for β-antigen
cyl	Encode for β-hemolysin
glnA	Encode for glutamine synthetase
cfb	Encode for the Christie–Atkins–Munch–Peterson (CAMP) factor
hylB	Encode for hyaluronidase
scaA	Encode for aggregation factor
bca	Encode for α-antigen
scpB	C5a peptidase
lmb	Laminin-binding protein
CLSI	Clinical Laboratory Standards Institute
PCR	Polymerase chain reaction

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Introduction

Cockroaches are considered among the most common pests in numerous homes and public places such as hospitals, hotels, bughouses, boarding schools, barracks, kindergartens, and dorms (Abdolmaleki et al. 2019b). Pest cockroaches are in close contact with humans (Doosti et al. 2015; Abdolmaleki et al. 2019a). Initially, they are tropical; however, most species live in parts of houses and other places where moisture, warmth, and food are adequate in the temperate zones (Feizhaddad et al. 2012; Abdolmaleki et al. 2019b). Among over 3500 recognized species, only a few ones are important to human, including *Blattella germanica* (German cockroach), *Periplaneta americana* (American cockroach), and *Blatta orientalis* (Oriental cockroach) (Pai et al. 2004; Lemos et al. 2006; Fakoorziba et al. 2010; Tetteh-Quarcoo et al. 2013; Pomés and Arruda 2014).

Cockroaches quickly move from buildings, gardens, drains, sewers, and latrines to human habitations. Since they feed on human food and feces, they can spread several types of pathogenic microorganisms. Likewise, several epidemiological investigations indicated that cockroaches were one of the main sources of different types of dangerous bacteria such as *Shigella dysenteriae*, *Salmonella typhi*, Streptococcus species (*spp*.), *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Pai et al. 2004; Lemos et al. 2006; Fakoorziba et al. 2010; Tetteh-Quarcoo et al. 2013; Pomés and Arruda 2014).

Streptococci are anaerobic gram-positive cocci forming a heterogeneous group with more than 30 different species. Some species such as *S. pneumoniae*, *S. pyogenes* (Lancefield Group A) and *S. agalactiae* (Lancefield Group B) are important human pathogens (Musher 1992; Tunkel and Sepkowitz 2002; Edwards and Baker 2005; Krzyaściak et al. 2013; Stevens and Bryant 2016; Ruppen et al. 2018). *S. pneumoniae* is recognized as one of the main causes of pneumonia, septic arthritis, acute sinusitis, endocarditis, pericarditis, peritonitis, meningitis, and septicaemia (Musher 1992; Edwards and Baker 2005; Krzyaściak et al. 2013). *S. pyogenes* causes serious infections including sepsis, necrotizing fasciitis, tonsillitis, and superficial skin infections (Edwards and Baker 2005; Krzyaściak et al. 2013; Stevens and Bryant 2016). Lastly, *S. agalactiae* is the most common cause of neonatal sepsis and animal mastitis (Krzyaściak et al. 2013; Ruppen et al. 2018).

Streptococcus spp. are mainly resistant to several antibiotics. In this regard, documented data indicated that Streptococcus spp. isolated from various clinical infections harbored a high prevalence of resistance against different antibiotics such as aminoglycosides, ampicillin, cephalothin, fluoroquinolone, sulfonamides, tetracyclines, trimethoprim, gentamicin, and chloramphenicol (Lin et al. 2004; Cattoir 2016). Studies into molecular epidemiological demonstrated that presence of specific antibiotic resistance genes, including those encoding resistance against penicillins (*pbp* (penicillin-binding protein)), tetracyclines (*tetK*, *tetM*, *tetO,* and *tetL*), macrolides (*erm* ((erythromycin ribosome methylase) and *mef* (macrolide efflux)), streptogramins A and B (*rplV*) (L22 and L4 ribosomal protein gene) and *lytA* (autolysin-encoding gene) are the most important reason for the occurrence of antibiotic resistance in these bacteria (Sapkota et al. 2006; Cattoir 2016).

Bac (encode for β-antigen), *cyl* (encode for β-hemolysin), *glnA* (encode for glutamine synthetase), *cfb* (encode for the Christie–Atkins–Munch–Peterson (CAMP) factor), *hylB* (encode for hyaluronidase), *scaA* (encode for aggregation factor), *bca* (encode for α-antigen), *scpB* (C5a peptidase) and *lmb* (laminin-binding protein) are another important virulence factors amongst the Streptococcus spp. isolated from clinical specimens (Kayansamruaj et al. 2014; Blumental et al. 2015). The presence of these virulence factors contributes to the high pathogenicity of Streptococcus spp. (Kayansamruaj et al. 2014; Blumental et al. 2015).

According to the high presence of cockroaches in the hospital environment as well as their significant importance as risk factors for maintenance and transmission of pathogenic bacteria, the present investigation was conducted to study the prevalence rate, distribution of virulence factors, and antimicrobial resistance properties of *S. pneumoniae*, *S. pyogenes* and *S. agalactiae* strains in German, American and Oriental cockroaches of Iranian hospitals.

Results

The present survey was conducted to assess the prevalence and distribution of the Streptococcus species' virulence factors and antibiotic resistance properties isolated from German, American, and Oriental cockroaches. Table 1 presents the total distribution of Streptococcus spp. isolated from different types of hospital cockroaches. The prevalence of *S. pyogenes*, *S. agalactiae* and *S. pneumonia* strains among all the studied samples was 4.82%, 1.66%, and 6.96%, respectively. Among other cockroach species, *S. pneumonia* had a higher prevalence (9.45%). A statistically significant difference was found between types of cockroaches and the prevalence of Streptococcus spp. ($P < 0.05$).

Table 2 shows the distribution of streptococcal putative virulence factors in different types of studied cockroaches. We found that *cfb* (53.93%), *cyl* (52.8%), *scaa* (51.68%), and *glna* (50.56%) were the most commonly detected streptococcal virulence factors. The highest prevalence of virulence factors was found in American cockroaches. The most

Table 1 Total distribution of Streptococcus spp. isolated from different types of hospital cockroaches

Samples	No. samples	Prevalence of Streptococcus spp. (%)		
		S. pyogenes	*S. agalactiae*	*S. pneumonia*
German cockroaches	161	8 (4.96)	1 (0.62)	11 (6.83)
American cockroaches	285	11 (3.85)	5 (1.75)	22 (7.71)
Oriental cockroaches	140	8 (5.71)	4 (2.85)	6 (4.28)
Other species	74	5 (6.75)	1 (1.35)	7 (9.45)
Total	660	32 (4.84)	11 (1.66)	46 (6.96)

Table 2 Distribution of streptococcal putative virulence factors in different types of the studied cockroaches

Virulence factors	Cockroaches species (*n*)				
	German (*n* = 20)	American (*n* = 38)	Oriental (*n* = 18)	Other species (*n* = 13)	Total number (*n* = 89)
Bca	2 (10*)	2 (5.26)	1 (5.55)	1 (7.69)	6 (6.74)
Bac	1 (5)	–	–	–	1 (1.12)
ScpB	2 (10)	2 (5.26)	1 (5.55)	2 (15.38)	7 (7.86)
Lmb	6 (30)	9 (23.68)	3 (16.66)	2 (15.38)	20 (22.47)
Cyl	14 (70)	19 (50)	8 (44.44)	6 (46.15)	47 (52.80)
GlnA	15 (75)	20 (52.63)	6 (33.33)	4 (22.22)	45 (50.56)
Cfb	17 (85)	22 (57.89)	9 (50)	–	48 (53.93)
HylB	11 (55)	17 (44.73)	6 (33.33)	5 (38.43)	39 (43.82)
ScaA	15 (75)	24 (63.15)	6 (33.33)	1 (7.69)	46 (51.68)

*Percent

commonly detected virulence factors amongst the German, American, and oriental cockroaches were *cfb* (85%), *ScaA* (63.15%), and *cfb* (50.00%), respectively. Among other cockroach species, *Cyl* (46.15%) was the most prevalent virulence factor. A statistically significant difference was found between types of cockroaches and the prevalence of virulence factors ($P < 0.05$).

Table 3 depicts the distribution of penicillin, macrolides, tetracyclines, and streptogramins antibiotic resistance genes in the Streptococcus spp. isolated from different types of hospital cockroaches. We found that *pbp2b* (71.91%), *pbp2x* (58.42%), *mefA* (46.06%), *ermB* (46.06%) and *tetM* (46.06%) were the most commonly detected antibiotic resistance genes in the Streptococcal spp. isolated from different types of hospital cockroaches. *TetO* (6.74%), *DF-L22* (21.34%), and *DF-L4* (24.71%) had the lowest prevalence in the studied antibiotic resistance genes. The most commonly detected antibiotic resistance genes amongst the German, American, and oriental cockroaches were *pbp2b* (80%), *pbp2b* and *pbp2x* (68.42%), and *tetM* (77.77%), respectively. Among other cockroach species, *pbp2b* (76.92%) was the most prevalent antibiotic resistance gene. A statistically significant difference was found between cockroaches and the prevalence of antibiotic resistance genes ($P < 0.05$).

Table 4 presents the antibiotic resistance pattern of the Streptococcus spp. isolated from different types of hospital cockroaches. Streptococcal spp. harbored the highest prevalence of resistance against tetracycline (80.89%), trimethoprim (65.16%), and penicillin (56.17%), while harbored the lowest against chloramphenicol (3.37%), ciprofloxacin (19.10%), and nitrofurantoin (29.21%). *S. pyogenes* strains harbored the highest prevalence of resistance against tetracycline (84.37%), penicillin (81.25%), enrofloxacin (56.25%), and erythromycin (53.12%). *S. agalactiae* strains harbored the highest prevalence of resistance against trimethoprim (72.72%), tetracycline (72.72%), penicillin (63.63%) and

Table 3 Distribution of antibiotic resistance genes among the Streptococcus spp. isolated from different types of hospital cockroaches

Resistance genes	Cockroaches species (*n*)				
	German (*n* = 20)	American (*n* = 38)	Oriental (*n* = 18)	Other species (*n* = 13)	Total number (*n* = 89)
LytA	4 (20)	17 (44.73)	3 (16.66)	1 (7.69)	25 (28.08)
pbp1a	10 (50)	21 (55.26)	5 (27.77)	4 (30.76)	40 (44.94)
pbp2x	15 (75)	26 (68.42)	3 (16.66)	8 (61.53)	52 (58.42)
pbp2b	16 (80)	26 (68.42)	12 (66.66)	10 (76.92)	64 (71.91)
mefA	12 (60)	22 (57.89)	1 (5.55)	6 (46.15)	41 (46.06)
ermB	13 (65)	20 (52.63)	7 (38.88)	1 (7.69)	41 (46.06)
DF-L22	6 (30)	8 (21.05)	5 (27.77)	–	19 (21.34)
DF-L4	8 (40)	13 (34.21)	1 (5.55)	–	22 (24.71)
tetM	6 (30)	17 (44.73)	14 (77.77)	4 (30.76)	41 (46.06)
tetO	3 (15)	2 (5.26)	–	1 (7.69)	6 (6.74)
tetL	5 (25)	17 (44.73)	1 (5.55)	1 (7.69)	24 (26.96)
tetK	15 (75)	23 (60.52)	1 (5.55)	1 (7.69)	40 (44.94)

Table 4 Antibiotic resistance pattern of Streptococcus spp. isolated from different types of hospital cockroaches

Bacteria (No. positive)	Resistance pattern (%)											
	P	TET	LIN	ERY	ENR	CIP	W	SXT	CEF	CHL	NIT	GEN
S. pyogenes (32)	26 (81.25)	27 (84.37)	9 (28.12)	17 (53.12)	18 (56.25)	7 (21.87)	16 (50)	16 (50)	13 (40.62)	2 (6.25)	–	14 (43.75)
S. agalactiae (11)	7 (63.63)	8 (72.72)	6 (54.54)	7 (63.63)	5 (45.45)	1 (9.09)	8 (72.72)	4 (36.36)	4 (36.36)	1 (9.09)	–	2 (18.18)
S. pneumonia (46)	17 (36.95)	37 (80.43)	23 (50)	17 (36.95)	13 (28.26)	9 (19.56)	34 (73.91)	11 (23.91)	13 (28.26)	–	–	10 (21.73)
Total (89)	50 (56.17)	72 (80.89)	38 (42.69)	41 (46.06)	36 (40.44)	17 (19.10)	58 (65.16)	31 (34.83)	30 (33.70)	3 (3.37)	–	26 (29.21)

P Penicillin (10 µg/disk), *TET* Tetracycline (30 µg/disk), *LIN* Lincomycin 15 (µg/disk), *W* Trimethoprim (5 µg/disk), *SXT* Sulfamethoxazole (25 µg/disk), *ERY* Erythromycin (15 µg/disk), *ENR* Enrofloxacin (5 µg/disk), *CIP* Ciprofloxacin (5 µg/disk), *CEF* Cephalothin (30 µg/disk), *CHL* Chloramphenicol (30 µg/disk), *GEN* Gentamicin (10 µg/disk), *NIT* Nitrofurantoin (100 µg/disk)

erythromycin (63.63%). *S. pneumoniae* strains harbored the highest prevalence of resistance against tetracycline (80.43%), trimethoprim (73.91%), and lincomycin (50.00%).

Discussion

Medically, cockroaches are much more important than generally realized as they have been demonstrated to harbor some pathogenic and non-pathogenic microorganisms. Since various workers have reported the isolation of various human pathogens from these insects, cockroaches are known vectors of human enteropathogens. Their filthy and nocturnal habits cause them to be ideal carriers for transmitting numerous pathogenic microorganisms. Klebsiella spp., *E. coli*, *P. aeruginosa*, Streptococcus spp. and some other potential pathogens have been isolated from cockroaches gathered from hospitals (Pai et al. 2004; Lemos et al. 2006; Fakoorziba et al. 2010; Tetteh-Quarcoo et al. 2013).

The present investigation was conducted to assess the prevalence rate, antibiotic resistance pattern, and genotyping evaluation of antibiotic resistance and virulence factor of the Streptococcus spp. isolated from American, German, oriental, and other species of hospital cockroaches. We found that *S. pneumoniae* had the highest prevalence among the studied cockroaches (6.96%). Oriental cockroaches had the highest prevalence of *S. pyogenes* (5.71%) and *S. agalactiae* (2.85%), while German cockroaches had the highest prevalence of *S. pneumoniae* (6.83%). Easily transmission of Hospital cockroaches in different parts of hospitals and sewage systems caused a high prevalence of Streptococcus spp. According to the research, American cockroaches are the most dangerous cockroach in nosocomial infections as they carry both of the highest virulence factors and antibiotic-resistant genes. Various studies have been carried out in this field. For example, Fotedar et al. (1991) indicated that one hundred and fifty-eight out of 159 (99–4%) cockroaches gathered from the hospital (test) and 113 out of 120 (94–2%) cockroaches gathered from residential areas (control) carried medically significant microorganisms. They indicated that 10–20% of cockroaches harbored Streptococcus spp. Kassiri et al. (2014) disclosed that culturing outer surface wash of cockroaches resulted in the isolation of *Klebsiella,* Pseudomonas*, E. coli, Staphylococcus, Proteus*, and *Streptococcus*. The main common bacteria were *Klebsiella* (35%) and *Pseudomonas* (30%). Elgderi et al. (2006) indicated that 27 and 25 species of the potential pathogen were isolated from the hospital and household cockroaches, respectively, with *Klebsiella*, *Enterobacter*, *Serratia*, and *Streptococcus* being predominant. Salehzadeh et al. (2007) demonstrated that 130 out of 133 (98%) German cockroaches had contamination with a high bacterial load (more than 1×10^3). *Enterobacter* (22.60%), *Klebsiella* (21%), Enterococcus

(17.30%), *Staphylococcus* (16.50%), *E. coli* and *Streptococcus* (8.3%), *Pseudomonas* (3%), as well as *Shigella*, *Haemophilus* and group A ß-hemolytic *Streptococcus* (less than 1%) were the most commonly detected bacteria. Pai et al. (2004) revealed that the prevalence of *Streptococcus* spp. in the intestinal content and surface of American and German cockroaches were 38.10% and 38.80% and 32.80% and 17.20%, respectively. Similar findings were achieved in the studies conducted in Iran (Ahmad Vahhabi et al. 2011; Kassiri and Shahnaz 2012), Thailand (Chaichanawongsaroj et al. 2004), and Brazil (Prado et al. 2006). As *S. agalactiae* derived from animal species, particularly in the cases of bovine mastitis, its presence in the cockroaches may be explained by the permanent presence of cockroaches in the environment. They can transfer easily anywhere. As a result, they may transfer from the area near to mastitic cows.

Results of our investigation indicated that the Streptococcus spp. strains isolated from cockroaches harbored a high prevalence of resistance against the commonly used antibiotics, particularly tetracycline, trimethoprim, enrofloxacin, erythromycin, lincomycin, and penicillin. The findings demonstrate the antibiotic resistance seriousness of the common pathogenic bacteria in Iran. A boost prevalence of antibiotic resistance was also reported in the pathogenic bacteria in the hospitals of Taiwan (Chang et al. 2000). More than 30% of *S. pneumoniae*, *S. aureus*, Enterobacteriaceae, *P. aeruginosa*, *Acinetobacter baumannii*, *Haemophilus influenza*, coagulase-negative *Staphylococci*, beta-hemolytic *Streptococci*, viridans *Streptococci*, and *Enterococcal* isolates of Taiwanese hospitals were resistant to different antibiotics (Chang et al. 2000). Pai et al. (2004) reported that all common pathogenic bacteria (Streptococcus spp. *S. aureus* and *P. aeruginosa*) isolated from cockroaches harbored resistance against ampicillin, chloramphenicol, tetracycline, trimethoprim and sulfamethoxazole. Bouamama et al. (2010) reported that pathogenic bacterial strains isolated from American cockroaches in Spain harbored the high prevalence of resistance against ampicillin, amoxicillin-clavulanate, cefoxitin; gentamicin, cotrimoxazole, and ciprofloxacin antibiotics. Hammad and Mahdy (2012) reported the high prevalence of antibiotic resistance of Streptococcus spp. isolated from cockroaches against ampicillin, cephalothin, chloramphenicol, ciprofloxacin, gentamycin, nalidixic acid, tetracycline, trimethoprim and sulfamethoxazole. Different patterns of antibiotic resistance of pathogenic bacterial strains isolated from cockroaches have been reported from Bangladesh (Islam et al. 2016), Nigeria (Chakraborty et al. 2015), and India (Leshan Wannigama et al. 2013). Such differences in the prevalence of antibiotic resistance reported in different studies may be due to the differences in the idea of medical practitioners in antibiotic prescription, availability and expense of antibiotics, and the laws of various countries for an antibiotic prescription. Furthermore, the high prevalence of antibiotic resistance reported in the present study may be due to the irregular and unauthorized prescription of antibiotics. The phenotypic pattern of antibiotic resistance was supported by the genotypic profile of antibiotic resistance genes. We found that the genes encoding resistance against penicillins (*pbp*), tetracyclines (*tetK*, *tetM*, *tetO,* and *tetL*), macrolides (*erm* and *mef*), streptogramins A and B (*rplV*), and the *lytA* gene had considerable prevalence in the Streptococcus spp. strains isolated from hospital cockroaches. To the best of our knowledge, there existed no previously published data in this field in Iran. High prevalence of *pbp*, *tetK*, *tetM*, *tetO*, *tetL*, *erm*, *mef*, *rplV,* and *lytA* antibiotic resistance genes was reported in the Streptococcus spp. strains isolated from different hospital infections (Malhotra-Kumar et al. 2005; Zeng et al. 2006; Xu et al. 2010). Kargar et al. (2012) reported the high prevalence of *ermB, mefA, pbp1a, pbp2b* and *pbp2x* genes in the *S. pneumonia* strains isolated from different types of the hospital infections of hospitalized patients in the Intensive Care Unit (ICU) centers. Presence of these genes in the Streptococcus spp. caused their severe resistance against some specific antibiotics. Our findings also disclosed a higher incidence of the phenotypic profile of resistance to some antibiotic agents than genotypic profiles. This finding is maybe because the presence of antibiotic resistance genes is one of the known procedures for antibiotic resistance in bacteria. On the other hand, higher incidence of phenotypic resistance toward antibiotics may be supported by procedures other than the presence of antibiotic resistance genes.

The final part of the present research was focused on detecting putative virulence genes in the Streptococcus spp. strains isolated from different types of hospital cockroaches. We found that *bac*, *cyl*, *glnA*, *cfb*, *hylB*, *scaA*, *bca*, *scpB,* and *lmb* had considerable prevalence in Streptococcus. Spp. strains isolated from hospital cockroaches. To the best of our knowledge, there existed no previously published data in this field worldwide. The α-protein of protein C was encoded by *bac* and *bca* genes. This gene group helps bacteria to enter the host cells. Genes *bac* and *bca* were detected in 1.12% and 6.74% of bacteria, respectively. Eskandarian et al. (2015) reported that the *bca* and *bac* genes were found in 14.6% and 9.7% of Streptococcus isolates of hospital infections. A lower prevalence of the *bac* gene was reported from the United States, New Zealand, and Europe (Manning et al. 2006; Persson et al. 2008; Sadowy et al. 2010). We found that the prevalence of *cyl, lmb,* and *scpB* genes was 52.80%, 22.47% and 7.86%, respectively. Duarte et al. (2005) reported that the prevalence of *lmb* and *scpB* genes in the Streptococcus spp. strains isolated from clinical samples was 97.30% and 96.70%, respectively, which was higher than our findings. Franken et al. (2001) and Dmitriev et al. (1999) also reported a higher prevalence of these genes. *Cfb* gene is encoded by complement factor B facilitating the essential

component of the alternative course of complement activation. Factor B circulates in the blood as one chain polypeptide. This gene was also predominant in the Streptococcus spp. strains isolated from different hospital infections (Udo et al. 2013; Ding et al. 2016).

The current survey revealed that hospital cockroaches, mainly German and American types, may be sources and reservoirs of pathogenic and antibiotic-resistant Streptococcus spp. Thus, monitoring hospital cockroaches may be helpful to decrease the dissemination of virulent and resistant bacteria in the hospital environment.

Conclusions

In summary, the present study results disclosed the high prevalence of different cockroaches in the hospital environment with high content of *S. pyogenes*, *S. agalactiae,* and *S. pneumoniae* strains. High prevalence of resistance against the commonly used antibiotics with the considerable distribution of virulence and antibiotic resistance genes in the Streptococcus spp. strains isolated from hospital cockroaches pose a significant public health issue. The presence of multi-drug resistant strains increases the importance of the research. The present study shows the high importance of hospital cockroaches as dangerous reservoirs for harboring virulent and resistant Streptococcus strains in the hospital environment and their transmission to the human population. Moreover, with a considerable rate of medically significant virulent and resistant bacteria, cockroaches may cause bacterial epidemic disease in hospitals. The findings indicate a possible role for cockroaches in the epidemiology of nosocomial infections, particularly those caused by Streptococcus spp. However, further studies are required to find additional knowledge about the microbiological and epidemiological roles of the hospital cockroaches in the survival and transmission of virulent and antibiotic-resistant bacteria.

Materials and methods

Sample's collection

From July 2016 to July 2020, 660 hospital cockroach samples were randomly collected from different educational hospitals. The cockroaches were gathered using hand catch, sticky traps, and vacuum cleaner methods. For sampling, sterile hand-gloves were used. Separate clean and sterile plastic bags were utilized to transfer the collected cockroaches (Paul et al. 1992). Only whole and alive cockroaches were investigated in the study. The samples were immediately transferred to Biotechnology Research Center, Shahrekord Branch, Sharekord, Iran. The cockroaches were identified using reliable taxonomic keys by an expert in the Department of Entomology, Shahrekord University, Shahrekord, Iran (Burgess 1993).

Isolation and identification of Streptococcus spp.

Sterile normal saline (0.9%) (5 mL) (Merck, Germany) was added to each test tube, and the cockroaches were vigorously washed and transferred to the secondary sterile test tubes using sterile forceps. A loop full of each suspension was cultured on streptococcal selection broth (BD Biosciences, USA) and incubated at 37 °C for 6 h with 5% CO_2. After enrichment, the samples were streaked onto 5% sheep blood agar and incubated at 37 °C for 24–48 h with 5% CO_2. The suspected streptococcal colonies were purified on BHI agar (Merck, Germany). The cultures purified were tentatively identified based on Gram's staining and biochemical tests, including bile esculin hydrolysis, catalase, and oxidase. Species identification was carried out using specific biochemical tests, including hemolysis activity (*S. pneumonia* (alpha), *S. pyogenes* (beta) and *S. agalactiae* (beta)), resistance to bacitracin (*S. pneumonia* (resistant/sensitive), *S. pyogenes* (sensitive) and *S. agalactiae* (resistant)), resistance to sulfamethoxazole (*S. pneumonia* (-), *S. pyogenes* (resistant) and *S. agalactiae* (resistant)), resistance to optochin (*S. pneumonia* (sensitive), *S. pyogenes* (resistant) and *S. agalactiae* (resistant)), bile:asculin activity (*S. pneumonia* (−/−), *S. pyogenes* (−/−) and *S. agalactiae* (−/+ and −/+)) and growth on 6.5% NaCl (*S. pneumonia* (−), *S. pyogenes* (−) and *S. agalactiae* (−)). Confirmation of the species was carried out using the specific Polymerase Chain Reaction (PCR). According to the manufacturer's instruction, genetic DNA was extracted from bacterial colonies using the DNA extraction kit (Fermentas, Germany). Purity (A260/A280) and concentration of extracted DNA were then checked (NanoDrop, Thermo Scientific, Waltham, MA, USA). The truth of the DNA was assessed on a 2% agarose gel stained with ethidium bromide (0.5 µg/mL) (Thermo Fisher Scientific, St. Leon-Rot, Germany). Table 5 shows the list of primers and the PCR conditions used to detect *S. pyogenes*, *S. agalactiae,* and *S. pneumonia* in DNA samples. Primers specific for *S. pyogenes*, *S. agalactiae,* and *S. pneumonia* were designed based on its conservative 16S ribosomal RNA (16S rRNA) gene. Each PCR reaction contained 4 µL of the extracted template DNA, 1.25 U of Taq DNA polymerase, 1 µM of each primer, 2 mM $MgCl_2$, 5 µL of 10X PCR buffer, 200 µM dNTPs, and double-distilled water was added to a final volume of 25 µL.

Antibiotic susceptibility test

Antibiotic resistance patterns of Streptococcus spp. were specified by the simple disk diffusion method. The

Table 5 Oligonucleotide primers and PCR conditions used to detect virulence factors and antibiotic resistance genes in the Streptococcus spp

Gene	Primer sequence (5'–3')	Amplicon size (bp)	PCR program	References
S. pyogenes	GGTTTGATGGGGATAAGGTGC TGGAAGTTAAAGTGAGTTGTCTGC	370	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/61 s 55 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min	In this study
S. pneumonia	CTGTTACTTGTTCTGGACTCTCGA TAATTGG GCCCACTCCTGTTAAAATCCTACC CGCATTG	430	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/65 s 55 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Li et al. (2019)
S. agalactiae	TAGATGGCGAATTCACTGAGA ATTGAGCAATCCCTATCACG	112	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 60 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min	Naccache et al. (2018)
LytA	CAACCGTACAGAATGAAGCGG TTATTCGTGCAATACTCGTGCG	319	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/62 s 55 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Billal et al. (2008)
pbp1a	AAACAAGGTCGGACTCAACC ATATACATTGGTTTATAGTAAGTT	195	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 55 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min	Billal et al. (2008)
pbp2x	CCAGGTTCCACTATGAAAGTG ATCCCAACGTTACTTGAGTGT	203	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/61 s 57 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min	Billal et al. (2008)
pbp2b	CCTATATGGTCCAAACAGCCT GGTCAATTCCTGTCGCAGTA	147	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 58 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Billal et al. (2008)
mefA	CTGTATGGAGCTACCTGTCTGG CCCAGCTTAGGTATACGTAC	294	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 58 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Najafi Mosleh et al. (2014)
ermB	CGTACCTTGGATATTCACCG GTAAACAGTTGACGATATTCTCG	224	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/61 s 57 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min	Kasahara et al. (2005)
DF-L22	GAACTCAGCTGTAGCTAACGC TTCTGCAACAGCTACAGTGATG	176	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 57 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Farrell et al. (2003)
DF-L4	AGCGATGCAGTATTTGGTATCG GCCGTATGAACGTGGAGTTG	236	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 58 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Farrell et al. (2003)
tetM	GAACTCGAACAAGAGGAAAGC ATGGAAGCCCAGAAAGGAT	740	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 55 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Ding et al. (2016)
tetO	AACTTAGGCATTCTGGCTCAC TCCCACTGTTCCATATCGTCA	519	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 520C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Lopardo et al. (2003)
tetL	TGAACGTCTCATTACCTG ACGAAAGCCCACCTAAAA	993	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/61 s 500C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min	Lopardo et al. (2003)
tetK	TCCTGGAACCATGAGTGT AGATAATCCGCCCATAAC	189	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 50 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Lopardo et al. (2003)
Bac	TGTAAAGGACGATAGTGTGAAGAC CATTTGTGATTCCCTTTTGC	530	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 50 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Ding et al. (2016)
Bca	TAACAGTTATGATACTTCACAGAC ACGACTTTCTTCCGTCCACTTAGG	535	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 51 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Manning et al. (2006)
ScpB	CCAAGACTTCAGCCACAAGG CAATTCCAGCCAATAGCAGC	591	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 57 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Ding et al. (2016)

Table 5 (continued)

Gene	Primer sequence (5'–3')	Amplicon size (bp)	PCR program	References
Lmb	ACCGTCTGAAATGATGTGG GATTGACGTTGTCTTCTGC	572	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/61 s 51 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min	Ding et al. (2016)
Cyl	ACGGCTTGTCCATAGTAGTGTTTG AACGACACTGCCATCAGCAC	345	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 52 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Ding et al. (2016)
GlnA	ACGTATGAACAGAGTTGGCTATAA TCCTCTGATAATTGCATTCCAC	471	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 52 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Ding et al. (2016)
ScaA	ACGGTATCAACCTTGAAACTGG TCAGTGTTGATTTCCCAGATGTA	256	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 52 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Dmitriev et al. (2002)
HylB	ACAAATGGAACGACGTGACTAT CACCAATTGGCAGAGCCT	346	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 52 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min 72 0C/5 min	Ding et al. (2016)
Cfb	GTAGAAGCCTTAACAGATGTGATTG AGTTTTGATTTTGTATAGATGGTAGC	251	1 cycle: 94 0C/8 min. 32 cycle: 95 0C/60 s 60 0C/70 s 72 0C/2 min 1 cycle: 72 0C/8 min	In this study

Mueller–Hinton agar (Merck, Germany) media were used for the antibiotic susceptibility test. For this purpose, the principles proposed by the Clinical and Laboratory Standards Institute (CLSI) were used (Wayne 2015). A 0.5 McFarland concentration of bacteria was prepared and used in the disk diffusion. Susceptibility of Streptococcus spp. was tested against tetracycline (30 u/disk), penicillin (10 u/disk), cephalothin (30 µg/disk), gentamicin (10 µg/disk), ciprofloxacin (5 µg/disk), lincomycin (15 µg/disk), nitrofurantoin (300 µg/disk), enrofloxacin (5 µg/disk), sulfamethoxazole (25 µg/disk), trimethoprim (5 µg/disk), erythromycin (15 µg/disk) and chloramphenicol (30 µg/disk) antibiotic agents (Oxoid, UK). The inoculated plates were aerobically incubated at 37 °C for 18–24 h in an anaerobic atmosphere. Results were interpreted based on the instruction provided by CLSI (2015). *S. pyogenes* ATCC 12,384, *S. pneumonia* ATCC 6305, and *S. agalactiae* ATCC 27,956 were used as quality controls.

PCR-based detection of virulence factors

Table 5 represents the primer sequence and PCR conditions used to detect putative virulence factors in the Streptococcus spp. isolated from different types of hospital cockroaches. Each PCR reaction contained 5 µL of 10X PCR amplification buffer, 2 µL of extracted DNA, 1 U of Taq DNA polymerase, 1 µM of each primer, 2 mM $MgCl_2$, 200 µM dNTPs, and double-distilled water was added to a final volume of 25 µL.

PCR-based detection of antibiotic resistance genes

Table 5 represents the primer sequence and PCR conditions used to detect penicillin, macrolide, streptogramin, and tetracycline antibiotic resistance genes in the Streptococcus spp. isolated from different types of hospital cockroaches. Each PCR reaction contained 3 µL of extracted template DNA, 1 U of Taq DNA polymerase, 1 µM of each primer, 2 mM $MgCl_2$, 5 µL of 10X PCR buffer, 200 µM dNTPs, and double-distilled water was added to a final volume of 25 µL. Positive DNA of each targeted gene was used as positive, while sterile PCR grade water (Thermo Fisher Scientific, Germany) was used as negative controls.

Agarose gel electrophoresis

The PCR amplified products (10 µL) were subjected to electrophoresis on a 1.5% agarose gel in 1X TBE buffer at 80 V for 30–40 min stained with a solution of ethidium bromide (Fermentas, Germany) and examined under Ultra Violet illumination (Uvitec, UK) (Ranjbar and Chehelgerdi 2018; Sadeghifard et al. 2010; Ranjbar et al. 2018).

Statistical analysis

The data obtained from all the tests were entered into the Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA) to be analyzed. All the data were first presented to the Kolmogorov–Smirnov to study their distribution. In this respect, the statistical analysis was then conducted using

SPSS/20.0 software (SPSS Inc., Chicago, IL). *P*-values were calculated using Chi-square and Fisher's exact tests to find any significant relationship between Streptococcus spp. and their virulence factors and antibiotic resistance properties among different types of hospital cockroaches. The *P*-value less than 0.05 was considered statistically significant.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethical approval and consent to participate This Research was approved in the Baqiyatallah University of Medical Sciences, Tehran, Iran (Project Ref Number 96-91002480). Verification of this research project and the licenses related to the sampling process were approved by Prof. Reza Ranjbar (Approval Ref Number Med-96-91002480).

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