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A Study on Diversity Among Streptococci Associated with Different Disease Conditions in Various Hosts and Their Comparative Antimicrobial Susceptibility to Herbal and Conventional Antimicrobials

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Abstract

Streptococcal infections are rampant in humans and animals, and are becoming a big problem for health workers to handle such infections to offer a cure due to the emergence of antimicrobial resistance (AMR). This study aimed to understand the diversity among streptococci in different disease conditions, host range, and their antimicrobial susceptibility patterns. A total of 504 isolates of 26 species of streptococci isolated from referred clinical (459) and non-clinical (44) samples during the period of 2018 to 2023 at the Clinical Epidemiology laboratory of the Division of Epidemiology were subjected to antimicrobial susceptibility testing using 39 conventionally used and 15 herbal antimicrobials by disc diffusion assays. The most common *Streptococcus* species isolates associated with clinical infections were *S. pyogenes*, *S. anginosus*, *S. porcinus*, *S. dysgalactiae*, *S. pneumoniae*, *S. equi, S. agalactiae*, *S. bovis*, *S. uberis*, and *S. suis*, contributing to >63% of cases of the streptococcal infections. Clinical strains showed high levels of MHDR, MDR, macrolide, and vancomycin resistance detected in 58.82%, 73.42%, 36.82%, and 43.51% of the strains, respectively. The maximum MHDR, MDR, macrolide, and vancomycin resistance was detected in strains associated with abortions and dental infections, respectively, and it was the minimum in the isolates recovered from otitis and conjunctivitis cases. The most effective herbal antimicrobials were thyme oil and carvacrol, inhibiting >80% of streptococcal isolates tested in the study. The findings of the study suggest an urgent need for the continuous monitoring of the antimicrobial susceptibility of streptococci causing clinical infections for advising effective antimicrobial therapy.

Keywords

AMR; MDR; MHDR; *S. pyogenes*; *S. anginosus*; *S. porcinus*; *S. dysgalactiae*; *S. pneumoniae*; *S. equi*; *S. agalactiae*; *S. bovis*; *S. uberis*; *S. suis*

1. Introduction

Streptococcal infections have shown a rising trend in the past few years [1] after the COVID-19 pandemic [2]. For detection of streptococcal infections, several rapid tests have been developed in the past [3, 4], but their utility became questionable after the emergence of antimicrobial resistance. The commonly recommended antibiotics were often found ineffective in controlling the streptococci infections due to the emergence of drug resistance, posing a great challenge to clinicians [5-8]. Though the drug-resistance phenomena are common among different types of streptococci, the major emphasis is laid on erythromycin-resistant Group A streptococci (GAS) responsible for respiratory tract infections, clindamycin-resistant Group B streptococci (GBS) leading to invasive infections, and multidrug-resistant (against β-lactams, macrolides, and fluoroquinolone antibiotics) *S. pneumoniae* because of upward trends of their infections noticed in the last decade [5, 9]. Owing to emerging and fast-spreading drug resistance among streptococci, they were included in the Bacterial Priority Pathogens List (BPPL) of antibiotic-resistant bacteria by the WHO, which had also emphasized the need for continuous monitoring and development of newer therapeutics against them [10].

Streptococci, especially GAS, are the major cause of sore throat (strep-throat), pharyngitis, follicular tonsillitis (acute and chronic), peritonsillar abscesses, scarlet fever, rheumatic fever, impetigo, cellulitis, pneumonia, necrotizing fasciitis (flesh-eating disease), Kawasaki disease, erysipelas, streptococcal toxic shock syndrome (STSS), PANDAS syndrome (paediatric autoimmune neuropsychiatric disorder associated with streptococcal infections) a subset of obsessive disorders in children (ODC) or tic disorders in children (TDC) and certain forms of psoriasis (guttate) may also be related to beta-haemolytic streptococcal infections and a long-standing strep-throat may be cause of lethal rheumatic heart disease [1, 11, 12]. Streptococci belong to many species and are classified through Lancefield classification viz., Group B (*S. agalactiae*), Group C (*S. equi*, *S. equimilis*, *S. zooepidemicus*), and G streptococci (*S. canis, S. dysgalactiae*), Group D (*S. equinus, S. bovis*), and viridians group (*S. mutans*, *S. salivarius*, *S. constellatus*, *S. intermedius, S. suis, S. iniae*), Group E (*S milleri*, *S. mitis*, *S. mitior*), Group F (*S. anginosus*), Group G (S. canis), Group H (*S. sanguis*), group R, S (*S. solitaries*), and many are not classified and are designated as non-Lancefield group streptococci viz., *S. pneumoniae* (major cause of respiratory tract infections, pneumonia, meningitis, bacteremia, and otitis media) have also been identified as an important cause of several severe infections in human and animals [13, 14]. However, in animals, *S. pneumoniae* is non-invasive in immunocompetent animals, but animals have been used as models to study its pathogenesis and drug development [15, 16]. However, several pneumococcal strains causing invasive pneumococcal disease (IPD) are reported from companion animals [17].

Considering the importance of emerging drug-resistant streptococci and opportunistic streptococci, this study investigates the diversity of *Streptococcus* species in different hosts and their associated antimicrobial resistance profiles to inform treatment strategies.

2. Materials and Methods

Bacterial strains: Streptococci isolated from referred clinical (459) and non-clinical (44) samples from 2018 to 2023 at the Clinical Epidemiology laboratory of the Division of Epidemiology, ICAR-Indian Veterinary Research Institute, Izatnagar-243 122, India. The isolates in the repository were revived from the glycerol stocks and checked for purity on 5% sheep blood agar (Difco-BBL, USA) and identity was confirmed using key biochemical characteristics [18, 19]. In cases of non-confirmed identity using conventional methods, the matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) using Bacterial Identification System (Broker Daltonics, Germany) was also used as an adjunct method. All revived isolates along with reference strain (*S. equi* ssp. *equi* MTCC 3522, susceptible to all antibiotics) obtained from the Zoonotic Disease Laboratory of the Institute were maintained on brain heart infusion agar (Difco-BBL, USA) till tested for their antimicrobial susceptibility.

Antimicrobial susceptibility testing (AST): All strains were tested for their susceptibility using disc diffusion assay as per CLSI guidelines [20] including the antibiotics recommended for group A (ampicillin, oxacillin, clindamycin, erythromycin, cotrimoxazole), group B (cefepime, cefotaxime, ceftriaxone, doxycycline, ofloxacin, meropenem, tetracycline, vancomycin), group C (amoxicillin, amoxicillin + clavulanic acid, chloramphenicol, Ertapenem, imipenem, linezolid) and few more including amoxicillin+sulbactam, azithromycin, aztreonam, cefixime, cefotaxime+ clavulanic acid, cefoxitin, cefpodoxime, ceftazidime, ceftazidime+clavulanic acid, cephoperazone+sulbactam, gentamicin, lincomycin, minocycline, moxalactam, nitrofurantoin, piperacillin, piperacillin + tazobactam, spectinomycin, and tigecycline. All antimicrobial discs were procured from Difco-BBL (USA). For conducting AST, plates of Muller Hinton agar (MHA, Difco) with 5% sheep blood were used. Inoculums were made by suspending overnight grown *Streptococcus* colonies in sterile normal saline solution equivalent to a 0.5 McFarland standard. Plates were swab inoculated and test discs of antimicrobials were placed 2 cm apart, incubated at 35°C ± 2°C, and the zone of inhibition was measured in mm. For quality control, *S. equi* ssp. *equi* (MTCC 3522, susceptible to all antibiotics) was used.

2.1 Herbal antimicrobial susceptibility testing

Streptococcal strains were tested through disc diffusion assay on a 5% blood agar medium in a way similar to the antimicrobial described above. Herbal-antimicrobial discs were made loading 1 mg of test herbal preparations including ≥99% pure carvacrol, citral, cinnamaldehyde, cinnamon (*Cinnamomum verum*) oil, lemongrass (*Cymbopogon citratus*) oil from Sigma Aldrich, USA; agarwood (*Aquilaria malaccensis*) oil, ajowan (*Tachyspermum ammi*) oil, betel (*Piper betel*) leaf oil, holy basil (*Ocimum sanctum*) oil, marjoram (*Origanum majorana*) essential oil, patchouli (*Pogostemon cablin*) essential oil, Rosewood (*Dalbergia sissoo*) oil, Sandalwood (S*antalum album*) oil, and thyme (*Thymus vulgaris*) oil from Nagaland Fragrance Pvt. Ltd (Dimapur, India) and guggul (*Commiphora wightii*) oil (ICAR-National Institute of Secondary Agriculture, Namkum, Ranchi, India). Any measurable zone of growth inhibition around herbal discs was considered a susceptibility of the bacterium [21]. Multiple antimicrobial resistance (MDR) was defined as resistance to ≥3 antimicrobial classes of antimicrobials, and multiple herbal drug resistance (MHDR) was defined as resistance to ≥3 herbal antimicrobials. *Streptococcus equi* ssp. *equi* (MTCC 3522) strain was used as a control.

2.2 Statistical analysis

The data of all strains about their species, Lancefield group, origin, and antimicrobial susceptibility were analyzed using Microsoft Excel to determine the relationship of different attributes of streptococcal strains with their resistance to different antimicrobials. To determine the relationship, the Odds ratio, the Chi-square (χ^2) test, and Pearson correlations were calculated using Microsoft Excel® 2010.

3. Results

A total of 26 species of streptococci were associated with nine diverse groups of clinical conditions, ranging from mild sore throat to septicemic deaths. A total of 459 strains belonging to 26 species (Table 1) were found associated with abortions, bacteraemia may or may not be leading to septicemic deaths, conjunctivitis, dental infection, otitis (media and externa), gastrointestinal infections (GITI), genital tract infections, mastitis, respiratory tract infections (RTI), skin infections, and urinary tract infections (UTI). The most commonly associated species of streptococci with abortions, bacteraemia/ septicaemia, GITI, genital tract infections, mastitis, RTIs, skin infections, and UTIs were *S. pyogenes* (47.73 %), *S. anginosus* + *S. pyogeneses* (37.37 + 22.22 = 59.59%), *S. anginosus* (44.44%), *S. anginosus* + *S. pyogenes* (33.33 + 55.56 = 88.89%), *S. dysgalactiae* ssp. *equisimilis* + *S. pyogenes* (16.95 + 20.34 = 37.29%), *S. pneumoniae* + *S. pyogenes* (16.22 + 28.38 = 44.60%), *S. anginosus* + *S. pyogenes* (15.38 + 48.72 = 64.10%) and *S. anginosus* + *S. pyogenes* (34.04 + 19.15 = 53.19%), respectively. Of the 459 isolates of streptococci associated with different ailments, the majority (52.46%) was constituted by strains of only three species, namely *S. pyogenes* (25.50%), *S. anginosus* (21.68%), and *S. pneumoniae* (5.28%).

Table 1. Streptococci in the study, their source, associated ailments, multiple herbal drug resistance (MHDR), multiple antimicrobial drug resistance (MDR), and resistance to macrolides (erythromycin/azithromycin) and vancomycin

Ailments	Affected hosts	*Streptococcus* spp.	MHDR	MDR	Resistance to macrolides	Resistance to vancomycin
Abortions (44)	Buffaloes 3, cattle 38, bitch 1, goats 2	*S. agalactieae* 1, *S. bovis* 2, *S. defactivus* 2, *S. dysgalactiae ssp. dysgalactiae* 1, *S. dysgalactiae* ssp. *equisimilis* 1, *S. iniae* 4, *S. milleri* (*anginosus*) 4, *S. pneumoniae* 4, *S. porcinus* 1, ***S. pyogenes* 21**, *S. sanguinis* 2, *S. uberis* 1)	84.09	56.82	22.73	31.71

Table 1 Continued

Bacteraemia/ septicaemia death (99)	Buffalo 1, cattle 6, crow 1, deer 9, dogs 6, falcon 1, guinea pigs 3, hawks 2, horses 3, humans 3, hyena 2, leopard 1, lions 5, lorikeets 2, peacocks 3, pigs 15, pigeons 4, poultry birds 22, rhinos 2, tigers 8)	*S. agalactieae* 1, *S. bovis* 5, *S. canis* 1, *S. dysgalactiae* ssp. *equisimilis* 6, *S. iniae* 1, *S. macacae* 1, ***S. milleri* (anginosus) 37**, *S. pneumoniae* 8, *S. porcinus* 15, ***S. pyogenes* 22,** *S. rattus* 1, *S. uberis* 1)	71.00	79.00	42.00	42.67
Eye infections/ conjunctivitis (2)	Horses 2	*Streptococcus equi* ssp. *zooepidemicus* 2	50.00	50.00	0.00	0.00
Dental infection (3)	Humans 3	*S. milleri* (*anginosus*) 3	66.67	100.0 0	100.00	66.67
Ear infections/otorrhoea (8)	Dogs 7, elephant 1	*S. equi* ssp. *equi* 1, *S. equi* ssp. *zooepidemicus* 3, *S. phacae* 1, *S. porcinus* 1, *S. pyogenes* 2	25.00	62.50	25.00	14.29
Gastrointestinal tract infections (18)	Cattle 5, dogs 3, horses 3, humans 3, pigs 4	*S. dysgalactiae* ssp. *equisimilis* 1, *S. iniae* 2, ***S. milleri*** (anginosus) **8**, *S. phocae* 1, *S. pneumoniae* 1, *S. porcinus* 1, *S. salivaris* 1, *S. sanguinis* 3	55.56	83.33	50.00	43.75
Genital tract infections including metritis, endometritis, cervicitis (27)	Cattle 1, Bitches 25, mithun-cow 1	*S. dysgalactiae* ssp. *dysgalactiae* 1, *S. equinus* 1, ***S. milleri* 9**, *S. porcinus* 1, ***S. pyogenes* 15**	60.71	75.00	32.14	56.00
Mastitis (59)	Buffaloes 15, cows 43, woman 1	*S. agalactiae* 5, *S. bovis* 3, *S. dysgalactiae* ssp. *dysgalactiae* 5, ***S. dysgalactiae* ssp. *equisimilis* 10,** *S. intestinalis* 5, *S. milleri* (*anginosus*) 10, *S.* (*Gemella*) *morbilorum* 1, *S. phocae* 1, *S. pneumoniae* 2, ***S. pyogenes* 12,** *S. sanguinis* 1, *S. uberis* 4	45.76.	76.27	23.731	44.00
Respiratory tract infection (74)	Buffaloes 1, dogs 6, goats 3, horses 19, humans 41, pigs 4	*S. agalactiae* 1, *S. anginosus* 4, *S. dysgalactiae* ssp. *equisimilis* 3, *S. equi* ssp. *equi* 2, *S. equi* ssp. *zooepidemicus* 5, *S. milleri* (*anginosus*) 15, *S. mitior* 2, ***S. pneumoniae* 12,** *S. porcinus* 2, ***S. pyogenes* 21,** *S. salivaris* 1, *S. sanguinis* 1, *S. suis* 5	45.95	72.97	40.54	51.67
Skin infections (78)	Buffalo 4, cattle 4, dogs 24, elephants 7, goats 2, horses 22, humans 4, mules 4, pigs 7	*S. agalactiae* 5, *S. anginosus* 1, *S. canis* 1, *S. dysgalactiae ssp. equisimilis* 8, *S. equi* ssp. *equi* 1, *S. equi* ssp. *zooepidemicus* 4, ***S. milleri*** (*anginosus*) **12,** *S. pneumoniae* 2, *S. porcinus* 5, ***S. pyogenes* 38**, *S. suis* 1	51.28	67.95	37.18	42.59

Table 1 Continued

Urinary tract infections (47)	Buffalo 1, cattle 1, dogs 11, horses 5, humans 23, lions 5, mule 2	*S. adjascens* 1, *S. agalactiae* 3, *S. alactolyticus* 3, *S. canis* 2, *S. dysgalactiae* ssp. *equisimilis* 5, *S. equi* ssp. *zooepidemicus* 3, ***S. milleri*** (*anginosus*) **16**, *S. mitis* 1, *S. porcinus* 4, ***S. pyogenes* 9**	65.96	78.72	46.81	42.50
All clinical (459)	Buffalo 25, cattle 97, crow 1, deer 9, dogs 83, elephants 8, falcon 1, goats 7, guinea pigs 3, hawks 2, horses 54, humans 78, hyena 2, leopard 1, lions 10, lorikkets 2, mithun 1, mules 5, peacocks 3, pigs 30, pigeons 4, poultry 22, rhinos 2, tigers 8	26 species of streptococci	58.82	73.42	36.82	43.512

Clinical strains had MHDR, MDR, macrolide (erythromycin/ azithromycin) resistance, and vancomycin resistance in 58.82%, 73.42%, 36.82%, and 43.51% of the strains. However, maximum MHDR, MDR, macrolide, and vancomycin resistance was detected in strains associated with abortions and dental infections (Table 1). It was evident that the higher the MDR value, the lower the number of isolates (Figure 1). The minimum levels of MDR, macrolide resistance, and vancomycin resistance were evident in strains associated with otitis (25%), conjunctivitis (50%), and conjunctivitis (0%), respectively.

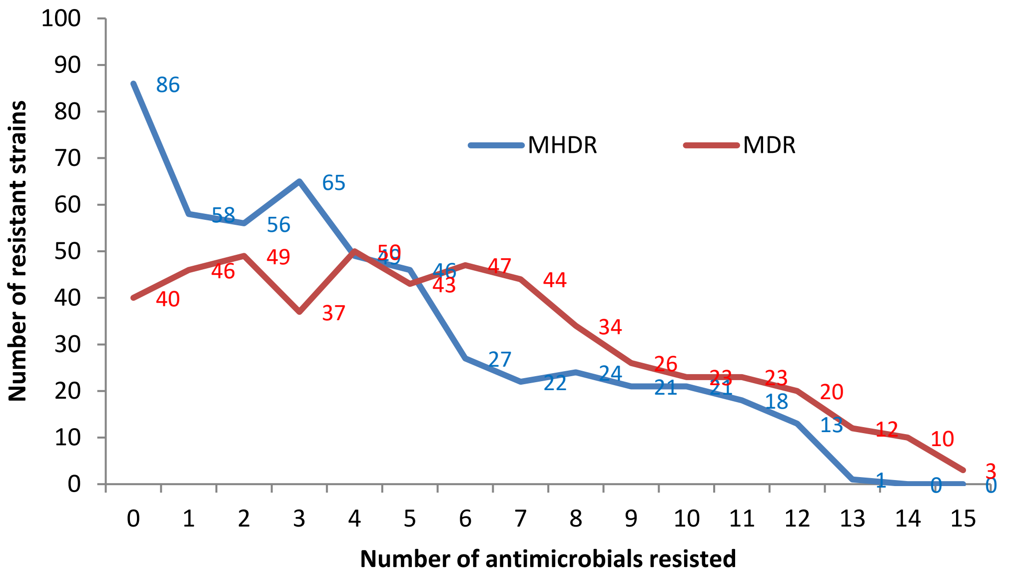

Figure 1. Trends of multiple herbal drug resistance (MHDR) and multiple antimicrobial drug resistance (MDR) among streptococci isolated from clinical and environmental samples from Bareilly region, India.

Among streptococci belonging to different Lancefield groups, group A (*S. pyogenes*) and group E, F (*S. anginosus* and *S. mitis*) dominated in clinical samples and were associated with eight and nine types of infections, respectively followed by group C, group G, H, group D and group B streptococci associated with 9, 8, 6, and 6 types of infection groups (Table 2). Other Lancefield non-classified strains belonging to 11 species were detected in association with all nine kinds of infections. Though *Streptococcus pneumoniae* was more commonly associated with respiratory tract infections, it was also detected in the blood of bacteraemia cases, faeces of GITI cases, milk of mastitic animals, and pus swabs of skin infection cases (Table 2).

Table 2. Antimicrobial drug susceptibility of streptococci of different Lancefield groups (if the number of antimicrobials with the same efficacy is more than two, names of all effective antimicrobials are given)

Streptococci and their traits	**Group A** streptococci (n=141)	**Group B** Streptococci (n=16)	**Group C** Streptococci (n=56)	**Group D** Streptococci (n=19)	**Group E-F** streptococci (n=154)	**Group G-H** streptococci (n=23)	**Non-Lancefield group** streptococci	**Other** streptococci (n=66)	**All** streptococci (N=504)
Species	*S. pyogenes*141	*S. agalactiae* 16	*S. dysgalactiae* ssp. *equisimilis* 34, *S. equi* ssp. *equi* 5, *S. equi* ssp. *zooepidemicus* 17	*S. bovis* 11, *S. equinus* 1, *S. suis* 7	*S. anginosus* (*S. milleri*) 149*, S. mitis* (*S. mitor*) 5	*S. canis* 4, *S. dysgalactiae* ssp. *dysgalactiae* 7, *S. sanguinus* 7	*S. pneumoniae* 29	11 species	26 species
Ailments associated	Abortions 21, bacteraemia 22, ear infection 2, genital tract infection 15, mastitis 12, RTI 21, skin infections 38, UTI 9, HB leaves 1	Abortion 1, bacteraemia 1, mastitis 5, RTI 1, skin infections 5, UTI 3	Abortions 1, bacteraemia 6, conjunctivitis 2, otitis 4, GITI 1, mastitis 10, RTI 10, skin infections 13, UTI 8	Abortion 2, bacteraemia 5, mastitis 3, skin wounds 2, pneumonia 3, sore throat 2, cow milk 1	Abortions 4, bacteraemia 37, dental infections 3, GITI 8, genital tract infections 9, mastitis 10, RTI 21, Skin infections 13, UTI 17, healthy buffalo milk 9, healthy elephant faces 1, human finger swab 1, healthy human urine 10, BAS scanner 7, HB leaves 3, pond water 1	Abortion 3, bacteraemia 1, GITI 3, mastitis 6, metritis 1, RTI 1, skin infection 1, UTI 2	Abortions 4, bacteraemia 8, GITI 1, mastitis 2, RTI 12, skin infections 2	8, Abortions 8, bacteraemia 19, ear infections 2, GITI 5, genital tract infection 1, mastitis 6, RTI 3, skin infections 5, UTI 8, healthy human urine 7, faeces of healthy pig and lion one each	455 clinical, 44 non-clinical, 5 references
Two best Herbal antimicrobials	Carvacrol, CNH	Carvacrol, AO, CO	RWO, Carvacrol	AO, Carvacrol, CNH	Carvacrol, TO	AO, Carvacrol, RWO	Carvacrol, TO	Carvacrol, TO	Carvacrol, TO
Two best conventional antimicrobials	Imp, Nf	AS, Ert, Imp, Pi, PiT	AS, Imp	Do, Min, Of	AS, Imp	AS, Imp	AS, Imp	AS, Imp	AS, Imp

Table 2 Continued

MHDR	59.57	25.00	42.86	57.89	66.88	60.87	62.07	69.70	60.32
MDR	58.87	75.00	67.86	84.21	79.22	82.61	89.66	81.82	73.41

Notes: GITI, gastrointestinal tract infections, RTI, respiratory tract infections, UTI, urinary tract infection; HB, holy basil; AO, ajowan oil; CNH, cinnamaldehyde; CO, cinnamon oil; TO, thyme oil; AS, amoxicillin + sulbactam; Do, doxycycline; Ert, ertapenem; Imp, imipenem; Min, minocycline; Nf, nitrofurantoin; Of, ofloxacin; Pi, piperacillin; PiT, piperacillin + tazobactam; MDR, multiple antibiotic drug-resistant; MHDR, multiple herbal antimicrobial-resistant; Penicillin group included oxacillin, ampicillin, amoxicillin + sulbactam, amoxicillin, amoxicillin + clavulanic acid, piperacillin, and piperacillin + tazobactam; Tetracycline group included tetracycline, doxycycline, minocycline, and tigecycline; Carbapenem group included Ertapenem, imipenem and meropenem.

Comparative susceptibility of streptococci associated with different ailments (Table 3) revealed that streptococci from samples from bacteraemia/ septicaemia, GITI, GTI, and UTI cases were significantly ($p \leq 0.05$) more often resistant to most of the antimicrobials than streptococci from abortion, mastitis, and RTI cases. However, streptococci from abortion cases were more often resistant to LGO and citral than streptococci associated with other ailments.

Table 3. Comparative antimicrobial susceptibility of streptococci isolated from clinical samples from various disease cases. (Only those species strains were compared where number of strains was ≥6)

Streptococci isolated from samples from cases of	Significantly ($p \leq 0.05$) more often resistant than isolates of streptococci associated with							
	Abortions	Bacteraemia/ septicaemia	Gastrointestinal tract infections	Genital tract infections	Mastitis	Respiratory tract infection	Skin infections	Urinary tract infections
Abortions	0	LGO, SWO	LGO, Citral, MHDR	LGO, MHDR	LGO, Citral, SWO, AWO, PO, GO, MHDR	LGO, Citral, SWO, AWO, GO, MHDR	LGO, SWO, GO, MHDR	LGO, SWO, MHDR
Bacteraemia/ septicaemia	AO, HBO, Carvacrol, MDR, Ox, Amx, AC, Pi, PG, T, Tet, G, Cot, Of, CTZ, CTZ-C, Cfx, CTX, CTX-C, CTR, Cfd, Cp, CPZ, Mox, Mp, Ert, CR, Az, E, Macro, Cd, Lin, Azt,	0	Citral	Cot, CTZ-C, Cfx, CTX, CTX-C, CFX, Mox, MP, Ert	AO, HBO, CHN, Carvacrol, LGO, TO, Citral, CO, SWO, PO, BLO, GO, MHDR, Ox, A, AC, PG, Nf, CTX, CTX-C, CTR, Cfd, CFX, Cp, Mox, Mp, Ert, CR, E, Macro, Lin	HBO, CHN, LGO, TO, Citral, CO, BLO, GO, RWO, Mo, MHDR, Amx, T, Do, Tig, Tet, G, CTZ, Cfx, CTX, CTX-C, CTR, Cfd, CFX, Cp, Mox, CFL, Mp, Ert, CR, Lin, Azt	AO, HBO, SWO, PO, GO, RWO, MHDR, PG, Nf, Cot, CTX, CTX-C, CTR, Cfd, Cp, CFL, Mp, Ert, Azt	Tig, CTR
Gastrointestinal tract infections	AO, HBO, MDR, Ox, Amx, AC, Pi, PiT, PG, T, Tet, G, Of, CTX, CTX-C, CTR, Cfd, Cp, Mox, Mp,Imp, Ert, CR, Az, E, Macro, Cd, Lin, Azt	Oxa, A, Pi, Of, Imp	0	Ox, Of, CTZ-C, Cfd, Mox, Mp, Cd	AO, HBO, BLO, Ox, A, AmS, AC, Pi, PiT, PG, T, Min, Tet, Of, CTR, Cfd, Mox, Mp, Imp, Ert, CR, Az, E, Macro, Cd, Lin	HBO, TO, BLO, Ox, A, AmS, Amx, Pi, PiT, T, Min, Tet, Of, CTX, CTR, Cfd, Cp, Mox, Mp, Imp, CR, E, Cd	HBO, Ox, A, AmS, Pi, PiT, T, Tet, Of, CTR, Cfd, Mox, Mp, Imp, CR, E, Cd	Ox, CTR

Table 3 Continued

Genital tract infections	AO, AmS, Amx, Pi, T, Tet, Cp, CPZ, Imp, CR, Az, V, Azt, Spt	0	0	0	HBO, Citral, Am-S, Imp, CR	HBO, To, Citral, Am-S, Amx, Tet, Cp, Imp	AmS	0
Mastitis	MDR, CTX, Azt	0	0	CTZ-C	0	RWO, Tig, CTX, Cp, Azt	Azt	Tig, Lz
Respiratory tract infection	AO, PG, Az, Macro, V	0	0	0	SWO, PO, Ox	0	0	0
Skin infections	Ox, AC, G, Of, CTZ-C, Cfx, CTX, CPZ, CR, Az, E	0	0	CTZ-C, Cfx	HBO, CNH, Carvacrol, LGO, Citral, BLO, Ox, AC, Cfx	HBO, To, Citral, BLO, Am-S, MO, Cfx, CTX	0	Tig, Lz
Urinary tract infections	AO, MDR, Ox, Amx, AC, Pi, PiT, PG, T, Do, Tet, G, Cot, Of, Cfx, CTX, CTX-C, Cfd, CFX, Cp, Mox, Mp, Imp, Ert, CR, Az, E, Macro, Cd, Lin, Azt	Of, Az	0	G, Cot, Of, CTZ-C, Cfx, CTX-C, Mp, Ert, Az	AO, HBO, CNH, Carvacrol, LGO, Citral, SWO, BLO, GO, MHDR, Ox, A, Am-S, AC, Pi, PiT, T, G, Of, Cfd, Cfx, Mp, Ipm, Ert, CR, Az, E, Macro, Lin	CNH, TO, Citral, BLO. GO, RWO, MO, MHDR, Am-S, T, Do, G, Cot, Of, CTZ, Cfx, CTX, CTX-C, Cfd, Cp, Mox, Mp, Imp, Ert, CR, Az, Lin, Azt	GO, RWO, Am-S, T, Cot, Of, Cfd, Mp, Imp, Ert, CR, Az	0

Notes: AO, ajowan oil; AWO, agar wood oil; BLO, betel leaf oil; CNH, cinnamaldehyde; Co, cinnamon oil; GO, guggul oil; HBO, holy basil oil; LGO, lemongrass oil; RWO, rose wood oil; SWO, sandal wood oil; To, thyme oil; AC, amoxicillin + clavulanic acid; Amx, amoxicillin; AS, amoxicillin + sulbactam; A, ampicillin; Az, azithromycin; Azt, aztreonam; CR, carbapenem resistance; Cp, cefepime; Cfx, cefixime; CTX, cefotaxime; CTX-C, cefotaxime+ clavulanic acid; Cx, cefoxitin; Cfd, cefpodoxime; CTZ, Ceftazidime; CTZ-C, ceftazidime + clavulanic acid; CTR, ceftriaxone; CFL, cephalosporin group; CPZ-S, cephoperazone + sulbactam; C, chloramphenicol; Cd, clindamycin; Cot, cotrimoxazole; Do, doxycycline; Ert, Ertapenem; E, erythromycin; G, gentamicin; Imp, imipenem; Lin, lincomycin; Lx, linezolid; Macro, macroloides; Mp, meropenem; Min, minocycline; Mox, moxalactam; Nft, nitrofurantoin; Of, ofloxacin; Ox, oxacillin; PG, penicillin group; Pi, piperacillin; PiT, piperacillin + tazobactam; Spt, spectinomycin; T, tetracycline; Tet, tetracycline group ; Tig, tigecycline; V, vancomycin; MDR, multiple antibiotic drug-resistant; MHDR, multiple herbal antimicrobial drug-resistant.

Comparative antimicrobial susceptibility analysis of isolates of streptococci belonging to different species of streptococci (Table 4) revealed that isolates belonging to *S. agalactiae, S. dysgalactiae, S. equi* and *S. uberis* isolates were significantly (p, ≤ 0.05) less often resistant to most of the antimicrobials than isolates belonging to other species especially *S. anginosus, S, pneumoniae* and *S. porcinus*.

Table 4. Comparative antimicrobial susceptibility of streptococci belonging to different species (only those species strains were compared where number of strains was ≥6)

Streptococcus species	Significantly (p, <0.05) more resistant than streptococci belonging to												
	S. agalactiae	*S. bovis*	*S. dysgalactiae* ssp. *dysgalactiae*	*S. dysgalactiae* ssp. *equisimilis*	*S. equi* ssp. *zooepidemicus*	*S. iniae*	*S. milleri* (*S. anginosus*)	*S. pneumoniae*	*S. porcinus*	*S. pyogenes*	*S. sanguinis*	*S. suis*	*S. uberis*

Table 4 Continued

S. agalactiae	0	0	Az, E	0	Car, Lin	Nf, Lin	PiT, Tig	0	0	Car, PiT, Tig, NF, Of	0	MHDR, Tig, Of, Lin	SWO, Lin
S. bovis	AO, BLO, MO, AmS, NF, CFX, Mox, CFL, Imp, Ert	0	HBO, AWO, GO, MHDR, Amx, PG, Cot, Ert	HBO, TO, AWO, GO, RWO, MHDR, Ert	GO, A, Amx, Cot, Imp, Ert	GO, Cot, Mp, Ert	AWO, GO, Cot, Ert	Ert	GO	GO, A, Amx, PG, Tig, Cot, Cp, CFL, Ert	Ert	GO, MHDR, Amx, Cot	SWO, GO, Ert
S. dysgalactiae ssp. dysgalactiae	0	0	0	0	0	NF	0	0	0	G, Nf	0	Min	0
S. dysgalactiae ssp. equisimilis	MDR, AC, Imp, Ert	CTX-C, V	Ox, Amx, PG	0	A, Amx, Imp	0	0	0	0	A, Amx, PG, Tet, G, Cot, Of, CTX-C, Imp, V	0	Amx, Do	Citral, CO, MHDR
S. equi ssp. zooepidemicus	PO, MDR, CFL, Mp	CPZ	AWO, PO, CPZ	PO	0	PO, Mp	PO, CTZ-C	0	0	0	PO	0	0
S. iniae	LGO, MDR, AC, CFL, V	V	AWO, Oxa, Cd	0	V	0	0	0	0	V. Cd	V	0	Cd
S. milleri (*S. anginosus*)	HBO, CNH, LGO,Citral, BLO, MO, MDR, AMX, AC, Pi, G, Cot, CTX-C, CFL, Mp, Ert	CO, CTX-C, V	HBO, LGO,Citral, Co, GO, MHDR, Ox, AMX, PG, Ert, Az, E, Macro, Lin	AO, HBO, CNH, LGO, TO,Citral, Co, BLO, GO, RWO, MHDR, Nf, Cfx, Cp, Lin	RWO, MDR, Ox, CTX, Ert, Lin	AO, Citral, CFX	0	Ox	0	AO, HBO, CNH, CO, MO, MDR, Ox, Amx, AC, PG, Tig, G, Nf, Cot, Of, C, CTX, CTX-C, CTR, CFX, Cp, Mox, CFL, Ert, CR, Macro, V	AO	AO, Citral, RWO, MO, MHDR, Do, Min	AO, CNH, SWO
S. pneumoniae	CNH, LGO,Citral, MO, MDR, AC, G, Cot, CFX, CFL, Ert	CTX-C, CPZ	AWO, GO, Amx, CPZ, Az, Macro, Lin	AO, CNH, LGO, CO, GO, NF, CFL	MDR, Amx, Of, CTR	AO, NF, CTR, CFX	0	0	0	CNH, GO, MDR, AC, NF, Cot, Of, CTX-C, CTR, CFX, CFL, Macro, Azt	AO	AO, Min, Of	AO, CNH

Table 4 Continued

S. porcinus	AO, HBO, CNH, LGO,Citral, PO, BLO, MO, MDR, Ox, AC, Min, Tet, G, Cot, CFX, Cfd, CFL, Ert, Azt	CO, Ox, T, CTX-C, V, Azt	HBO, LGO, Citral, CO, SWO, PO, MHDR, Ox, Amx, PG, Cp, E, Macro, Cd, Lin, Azt	AO, HBO, CNH, LGO, TO,Citral, Co, SWO, PO, MHDR, Cfd, Cfx, Cp, CFL, Lin	CO, MHDR, MDR, Ox, Amx, T, CTX, Cfd, CFX, Ert, Lin	AO, Citral, Do, CTX, CFX, Spt	PO, T, Tet, CTZ, Azt	SWO, Ox, T, Tet, C, CTX, Cfd	0	CNH, MDR, Ox, Amx, PG, Tig, Tet, G, Nf, Cot, Of, C, CTX, CTX-C, CTR, CFX, Cp, Mox, CFL, Ert, V, Cd, Azt	AO, CFX	AO, Citral, SWO, MHDR, T, Do, Min, Tet, Of, Cfd, Azt	AO, SWO, CTZ
S. pyogenes	LGO, Citral, PO, MO, MDR, G, Cfx, CFL, Mp	0	AWO, Lin	LGO, CO, PO, RWO, MHDR, Lin	Lin	0	PO	0	0	0	0	Citral, Do, Min	SWO
S. sanguinis	MDR, A, Amx, Pi, G, Cot, CFL, Mp	E	AWO, GO, Amx, Cot, Of, Az, E, Macro, Cd, Lin	CO, CFL, E	MDR, A, Amx, T, Cot, Of, Imp, E	Cot, Mp	Cot, Of	0	0	MDR, Amx, Tet, Cot, Of, Mox, CFL, E	0	Amx, Do, Cot, Of	Of
S. suis	MDR, Pi, CFL, Ert, V	V	Ox, Ert	Car	Car, Imp, Ert	Ert	0	0	0	Car, Ert, V	Ert, Macro	0	0
S. uberis	MDR, Pi, Cot, CFL, Ert	CTX-C	Citral, AWO, MHDR, Macro	Citral, CO, MHDR	0	Citral, Spt	0	0	0	0	Citral	Citral, MHDR	0

Notes: AO, ajowan oil; AWO, agar wood oil; BLO, betel leaf oil; Car, carvacrol; CNH, cinnamaldehyde; Co, cinnamon oil; GO, guggul oil; HBO, holy basil oil; LGO, lemongrass oil; RWO, rose wood oil; SWO, sandal wood oil; To, thyme oil; AC, amoxicillin + clavulanic acid; Amx, amoxicillin; AS, amoxicillin + sulbactam; A, ampicillin; Az, azithromycin; Azt, aztreonam; CR, carbapenem resistance; Cp, cefepime; Cfx, cefixime; CTX, cefotaxime; CTX-C, cefotaxime+ clavulanic acid; Cx, cefoxitin; Cfd, cefpodoxime; CTZ, Ceftazidime; CTZ-C, ceftazidime + clavulanic acid; CTR, ceftriaxone; CFL, cephalosporin group; CPZ-S, cephoperazone + sulbactam; C, chloramphenicol; Cd, clindamycin; Cot, cotrimoxazole; Do, doxycycline; Ert, Ertapenem; E, erythromycin; G, gentamicin; Imp, imipenem; Lin, lincomycin; Lx, linezolid; Macro, macroloides; Mp, meropenem; Min, minocycline; Mox, moxalactam; Nft, nitrofurantoin; Of, ofloxacin; Ox, oxacillin; PG, penicillin group; Pi, piperacillin; PiT, piperacillin + tazobactam; Spt, spectinomycin; T, tetracycline; Tet, tetracycline group ; Tig, tigecycline; V, vancomycin; MDR, multiple antibiotic drug-resistant; MHDR, multiple herbal antimicrobial drug-resistant.

On comparing the antimicrobial susceptibility of clinical and non-clinical streptococci (Table 5), it was evident that non-clinical strains were more often resistant to penicillins and vancomycin, but to a lesser extent to macrolides and vancomycin than streptococci from clinical samples, and had higher levels of MDR and MHDR. However, clinical strains were significantly more resistant to tetracycline ($p < 0.05$), and less often ($p < 0.05$) to AO, HBO, CNH, carvacrol, LGO, LGO, TO, CO, SWO, BLO, GO, Ox, Pi, Nf, CTX, CFX, Cp, Mox, Lz, Cd, and lincomycin than those isolated from non-clinical samples (Tables 6, 7).

Table 5. Source of Streptococci and their resistance (expressed in % of resistant isolates) pattern

Source of isolates	n	MHDR	MDR	Resistance to			
				Macro-loides	Vanco-mycin	Peni-cillins	Tetra-cyclines
Clinical	459	58.82	73.42	36.82	43.51	49.67	57.08
Non-clinical	44	77.27	75.00	34.09	50.00	54.55	38.64
References (*S. equi* ssp. *equi* 1)	1	100.00	0.00	0.00	0.00	0.00	0.00
Human-clinical	78	57.59	80.77	47.44	50.82	46.15	65.38
Human-non-clinical	17	58.82	52.94	29.41	17.65	23.53	52.94
Domestic animals clinical	130	60.77	68.46	26.92	44.14	39.23	51.54
Domestic animals non-clinical	10	80.00	80.00	50.00	87.50	80.00	10.00
Dogs (all were clinical isolates)	83	57.83	73.49	39.76	34.85	51.81	57.83
Wild and zoo animals	41	65.85	87.80	46.34	54.29	53.66	78.05
Non-domestic birds	13	69.23	69.23	38.46	50.00	53.85	69.23
Horses (54) and mules (5)	59	38.98	55.93	25.42	42.55	45.76	37.29
Pigs	31	67.74	80.65	32.26	28.57	74.19	48.39
Poultry birds (died of septicaemia)	22	72.76	82.36	68.18	66.67	86.36	72.73
Carnivores	108	59.26	77.78	42.59	38.10	54.63	64.81
Herbivores	221	56.56	67.42	28.05	46.24	42.99	47.96
Omnivores	158	62.66	77.22	45.57	42.86	55.06	61.39
All (459 clinical, 44 non-clinical, 1 reference)	504	60.32	73.41	36.51	44.01	50.00	55.36

Table 6. Herbal antimicrobial resistance in streptococci isolates of clinical and non-clinical origin

Human clinical strains, n = 78		Animal Clinical strains, n = 381		Non-clinical strains, n = 44	
Antimicrobials	% Resistant strains	Antimicrobials	% Resistant strains	Antimicrobials	% Resistant strains
Carvacrol	9.23	Carvacrol	5.05	Carvacrol	15.91
Thyme oil	16.95	Thyme oil	20.09	Patchouli oil	20.00
Cinnamon oil	22.03	Ajowan oil	22.69	Thyme	35.00
Cinnamaldehyde	22.41	Cinnamaldehyde	22.96	Ajowan oil	39.53
Ajowan oil	37.10	Cinnamon oil	26.24	Cinnamaldehyde	46.51
Guggul oil	38.46	Holy basil oil	44.53	Agarwood Oil	60.00
Holy basil oil	45.16	Betel leaf oil	48.33	Cinnamon oil	62.79
Sandalwood oil	52.38	Sandalwood oil	48.57	Holy basil oil	65.12
Citral	53.33	Citral	52.92	Citral	65.12
Lemongrass oil	55.07	Guggul oil	53.33	Rosewood Oil	68.42
Betel leaf oil	57.78	Rosewood Oil	55.13	Sandalwood oil	74.42
Agarwood Oil	62.96	Lemongrass oil	57.40	Betel leaf oil	75.00
Patchouli oil	63.33	Patchouli oil	62.22	Guggul oil	75.00
Rosewood Oil	66.67	Agarwood Oil	62.92	lemongrass oil	76.74
Marjoram oil	72.00	Marjoram oil	70.73	Marjoram EO	89.47

Table 7. The most effective 25 conventional antimicrobials and antibiotic resistance in streptococci isolates from different sources

Human clinical strains, n = 78		Animal Clinical strains, n = 381		Non-clinical strains, n = 44	
Antimicrobial	% Resistant strains	Antimicrobial	% Resistant strains	Antimicrobial	% Resistant strains
Amoxicillin +sulbactam	10.71	Imipenem	13.18	Amoxicillin +sulbactam	0.00
Imipenem	15.63	Amoxicillin + sulbactam	15.38	Imipenem	2.86
Meropenem	16.67	Nitrofurantoin	19.89	Tigecycline	8.33
Piperacillin +Tazobactam	20.75	Piperacillin Tazobactam	22.44	Carbapenem group	20.45
Nitrofurantoin	21.74	Ofloxacin	22.60	Amoxicillin +clavulanic acid	23.53
Piperacillin	22.64	Carbapenem group	23.62	Erythromycin	23.81
Ampicillin	25.81	Piperacillin	24.80	Ofloxacin	26.47
Amoxicillin +clavulanic acid	29.31	Meropenem	28.15	Amoxicillin	26.67
Tigecycline	31.82	Chloramphenicol	28.40	Meropenem	27.27
Linezolid	31.91	Amoxillin + clavulanic acid	29.05	Ampicillin	31.25
Amoxicillin	33.33	Ampicillin	30.15	Piperacillin + Tazobactam	36.36
Chloramphenicol	36.62	Ertapenem	30.52	Chloramphenicol	37.50
Cefepime	37.50	Clindamycin	30.73	Tetracycline group	38.64
Oxacillin	43.18	Linezolid	31.76	Azithromycin	40.74
Cefotaxime+ clavulanic acid	43.75	Tigecycline	32.18	Nitrofurantoin	42.86
Cefotaxime	44.00	Azithromycin	32.47	Cefoperazone + sulbactam	43.75
Clindamycin	44.44	Amoxicillin	33.84	Piperacillin	45.45
Gentamicin	46.38	Erythromycin	35.38	Tetracycline	48.48
Erythromycin	46.88	Cotrimoxazole	37.22	Doxycycline	50.00
Ceftazidime	50.00	Vancomycin	42.07	Gentamicin	50.00
Ertapenem	50.00	Oxacillin	42.51	Cotrimoxazole	50.00
Ceftriaxone	51.02	Lincomycin	43.09	Ceftazidime	50.00
Vancomycin	51.61	Gentamicin	44.75	Vancomycin	50.00
Ofloxacin	52.70	Tetracycline	46.02	Linezolid	51.61
Doxycycline	53.33	Cefotaxime + clavulanic acid	46.15	Minocycline	52.00

Herbal antimicrobial resistance and resistance to a few selected antibiotics (AO, HBO, CNH, carvacrol, LGO, TO, CO, SWO, BLO, GO, Ox, Pi, NF, CTX, CFX, CP, Mox, Lz, Cd, Lin) were significantly ($p < 0.05$) more prevalent among streptococci of environmental origin than those isolated from clinical cases (Tables 6, 7). Irrespective of the origin of strains, the carvacrol was the most effective (93.22%) and marjoram oil (MO) was the least inhibitory (25.98%) herbal compound on streptococci. However, resistance to tetracyclines was more often (OR 2.26, CI_{95} 1.15-408) detected in streptococci from clinical samples.

The most effective antibiotics on streptococci isolates irrespective of their origin were amoxicillin + sulbactam and imipenem, while for other antibiotics, susceptibility varied to a variable extent among strains of different origins (Table 7), of different species (Table 8), and belonging to different Lancefield groups (Table 9).

Table 8. The most effective antimicrobials on different *Streptococcus* species isolates (if the number of antimicrobials with the same efficacy were more than two names of all effective antimicrobials are given)

Bacteria	Disease cases	MHDR	MDR	The two most effective herbal antimicrobials	The two most effective antibiotics
S. adjascens (2)	UTI 1	100.00	100.00	Carvacrol, TO	A, Amx, AS, G, Tig
S. agalactiae (16)	Abortion 1, bacteremia 1, mastitis 5, RTI 1, skin infections 5, UTI 3	25.00	75.00	Carvacrol, CO	AS, Pi, PiT
S. alactolyticus (3)	UTI 3	100.00	66.67	Carvacrol, CO, GO, LGO, PO	A, AC, AS, C, Nf, Pi, PiT, Tig
S. milleri (*S. anginosus*) (149)	Abortions 4, Bacteremia 37, dental infections 3, GITI 8, genital tract infections 9, mastitis 10, RTI 19, Skin infections 13, UTI 16, healthy buffalo milk 9, human finger swab 1, healthy human urine 10, BAS scanner 7, HB leaves 2, pond water 1	65.77	78.52	Carvacrol, TO	Imp, PiT
S. bovis (11)	Abortion 2, bacteremia 5, mastitis 3, cow milk 1	81.82	81.82	AO, BLO, Carvacrol, Citral, CNH, TO	Do, CTX-C, CPZ-S
S. canis (4)	Bacteremia 1, skin infection 1, UTI 2	75.00	75.00	AO, Carvacrol, RWO, SWO, TO	Tig, Nf
S. defactivus (4)	Abortions 2, healthy human urine 2	50.00	0.00	AO, Carvacrol, Citral, CNH, CO, HBO, TO	A, AC, Amx, AS, G, Tig
S. dysgalactiae ssp. *dysgalactiae* (7)	Abortion 1, genital tract infection 1, mastitis 5	28.57	85.71	AO, Carvacrol, CNH, CO	AC, Amx, AS, Az, Cd, E, Lin, Pi, PiT
S. dysgalactiae ssp. *equisimilis* (34)	Abortion 1, bacteremia 6, GITI 1, mastitis 10, RTI 3, skin infections 8, UTI 5	41.18	76.47	CO, RWO	Min, Nf
S. equi ssp. *equi* (5)	Ear infection 1, RTI 2, skin infection 1, reference 1	40.00	60.00	AO, Carvacrol,	A, AC, AS, Pi, PiT, Ox,CTR
S. equi ssp. *zooepidemicus* (17)	Conjunctivitis 2, ear infections 3, RTI 5, skin infections 4, UTI 3	47.06	52.94	AO, Carvacrol, CNH, CO, RWO	AS, Ert, Ipm, Min
S. equinus (1)	Genital tract infection 1	0.00	100.00	AO, Carvacrol, Citral, CNH, CO, LGO, TO	C, Do, G, Nf, Min, Of, T, Tig
S. iniae (8)	Abortions 4, Bacteremia 1, GITI 2, healthy pig 1	62.50	75.00	AO, Carvacrol, TO	As, CTX-C, Do, Lin
S. intestinalis (5)	Mastitis 5	80.00	60.00	LGO, GO	A, Amx, Cot, Of
S. macacae (1)	Bateremia 1	0.00	0.00	AWO, GO, SWO	A, Amx, C, Cot, G, Nf, Of
S. mitis (5)	RTI 2, UTI 1, HB leaves 1, healthy elephant faeces 1	100.00	100.00	Carvacrol, CNH	AS, Min, Lz
S. morbilorum (*Gemella morbilorum*)	Mastitis 1	0.00	100.0	All herbal antimicrobials	All antibiotics except Ox, Cfx, Az, Azt
S. phocae (4)	Ear infection 1, GITI 1, mastitis 1, healthy human urine 1	50.00	100.00	Carvacrol, TO	AC, Amx, AS, Ipm, Lz, Pi, PiT
S. pneumoniae (29)	Abortions 4, bacteremia 8, GITI 1, mastitis 2, RTI 12, skin infections 2	62.07	89.66	Carvacrol, TO	Ipm, As, Mp
S. porcinus (34)	Abortions 1, bacteremia 15, ear infection 1, GITI 1, genital tract infection 1, RTI 2, skin infections 5, UTI 4, healthy human urine 4	76.47	91.18	Carvacrol, TO	AS, Ipm

Table 8 Continued

S. pyogenes (141)	Abortions 21, bacteremia 22, ear infection 2, genital tract infection 15, mastitis 12, RTI 21, skin infections 38, UTI 9, HB leaves 1	59.57	58.87	Carvacrol, CNH	Ipm, NF
S. rattus (1)	Bacteremia 1	100.00	100.00	Carvacrol	AC, Amx, AS, Ipm, Lz, Pi, PiT
S. salivaris (2)	GITI 1, RTI 1	0.00	100.00	AO, Carvacrol, Citral, CO, CNH, LGO, TO	AS, Az, CTR, E, G, Ipm, Mp, NF
S. sanguinis (7)	Abortion 2, GITI 3, mastitis 1, TRI 1	71.43	100.00	AO, Carvacrol, CNH	AC, C, Nf
S. suis (7)	RTI 5, skin infection 1, pond water 1	28.57	85.71	AO, CO, CNH, Citral, TO	Amx, Do, Min,
S. uberis (7)	Abortion 1, bacteremia 1, mastitis 4, healthy lion faeces 1	85.71	85.71	AO, CNH, TO	AS, Ipm

Notes: GITI, gastrointestinal tract infections; RTI, respiratory tract infections; UTI, urinary tract infections; AO, ajowan oil; AWO, agar wood oil; BLO, betel leaf oil; CNH, cinnamaldehyde; Co, cinnamon oil; GO, guggul oil; HBO, holy basil oil; LGO, lemongrass oil; RWO, rose wood oil; SWO, sandal wood oil; To, thyme oil; A, ampicillin; Amx, amoxicillin; AC, amoxicillin + clavulanic acid; AS, amoxicillin + sulbactam; Az, azithromycin; C, chloramphenicol; Cd, Clindamycin; Cot, contrimoxazole; CPZ-S, cefoperazone + sulbactam; CTR, ceftriaxone; CTX-C, cefotaxime + clavulanic acid; Do, doxycycline; E, erythromycin; Ert, ertapenem; G, gentamicin; Imp, imipenem; Lin, lincomycin; Mp, meropenem; Lz, linezolid, Min, minocycline; Nf, nitrofurantoin; Of, ofloxacin; Ox, oxacillin; Pi, piperacillin, PiT, piperacillin + tazobactam; T, tetracycline; Tig, tigecycline.

Table 9. The best and worst antimicrobials for streptococci of different groups

Lancefield Group	Species	Inhibiting ≥80% of strains		Inhibiting <50% strains	
		Antibiotics	Herbals	Antibiotics	Herbals
A	*S. pyogenes* 141	Imp, Nf, AS, Ert, Of, AC, PiT, Tig, Pi	Carvacrol, CNH	Do, CTZ-C, Azt, CTZ, Cfd, Cfx, Spt, Lin	LGO, Citral, Mo, SWO, RWO, AWO, PO
B	*S. agalactiae* 16	Imp, AS, Ert, PiT, Pi, Mp, Nf, Of, AC, Cd, Ox, Lz, Az	Car, AO, CNH, CO, TO, HBO, Citral	T, Cfd, Spt, Cfx, Azt, CPZ-S	PO, MO, AWO
C	*S. dysgalactiae ssp. equisimilis 34, S. equi* ssp. *equi 5, S. equi ssp. zooepidemicus* 17	AS, Imp, Nf	RWO, carvacrol, CO, AO, TO, CNH	CTX-C, Cfx, Cfd, CPZ-S, Mox, CTZ, CTZ-C, Azt, Spt	Awo, PO, MO
D	*S. bovis* 11, *S. equinus* 1, *S. suis* 7	Do, CTX-C, Min, Of	AO, Carvacrol, CNH, CO	CTX, CTR, Mox, Amx, Azt, CTZ-C, CFX, CTZ, Spt, Cot, Cp, Ert, Cfd	PO, HBO, GO, AWO
E-F	*S. anginosus* (*S. milleri*) 149, *S. mitis* (*S. mitor*) 5	Imp	Carvacrol	V, CPZ-S, CTZ, Do, G, Min, Ox, CTR, Lin, CTX-C, Cp, CTX, Cfd, CFX, Mox, Azt, Spt	SWO, AWO, HBO, GO, BLO, citral, LGO, RWO, MO
G-H	*S. canis* 4, *S. dysgalactiae* ssp. *dysgalactiae* 7, *S. sanguinus* 7	AC, Ert	Carvacrol, AO, RWO, CNH, TO, CO	CFX, T, CTR, CTZ-C, CTZ, Cx, G, Cot, Cfd, Mox, Azt, Spt, Min	GO, AWO, PO, MO
Non-lancefield group	*S. pneumoniae* 29	Imp	Carvacrol, TO,	G, Lin, CTX-C, Cfd, CTZ, Mox, Min, CPZ-S, CTR, CFX, Spt, Azt	Citral, LGO, AWO, MO, GO
Others	11 species 66 strains	AS, Imp, Mp	Carvacrol, TO	Do, Cfx, Cx, CTR, Min, T, CPZ-S, Ox, CTX, Cp, Mox, CTZ-C, CTZ, Cfd, Azt, Spt	Citral, SWO, BLO, LGO, MO, PO, AWO, RWO

Table 9 Continued

All	26 species 504 strains	AS, Ipm	Carvacrol	Do, Min, CTZ, CTZ-C, Cfx, CTX, CTR, Cfd, Cx, Cp, CPZ-Smox, Azt, Spt	BLO, LGO, Citral, Mo, SWO, RWO, AWO, PO
Clinical	26 species	AS, Ipm	Carvacrol, TO	Do, CTR, CTX, CPZ-S, Min, Mox, Cfx, Cx, CTZ-C, CTZ, Cfd, Azt, Spt	GO, Citral, LGO, RWO, PO, AWO, MO

Notes: AO, ajowan oil; AWO, agar wood oil; BLO, betel leaf oil; CNH, cinnamaldehyde; Co, cinnamon oil; GO, guggul oil; HBO, holy basil oil; LGO, lemongrass oil; RWO, rose wood oil; SWO, sandal wood oil; To, thyme oil; A, ampicillin; Amx, amoxicillin; AC, amoxicillin + clavulanic acid; AS, amoxicillin + sulbactam; Az, azithromycin; C, chloramphenicol; Cd, Clindamycin; Cot, contrimoxazole; CPZ-S, cefoperazone + sulbactam; CTR, ceftriaxone; CTX-C, cefotaxime + clavulanic acid; Do, doxycycline; E, erythromycin; Ert, ertapenem; G, gentamicin; Imp, imipenem; Lin, lincomycin; Mp, meropenem; Lz, linezolid, Min, minocycline; Nf, nitrofurantoin; Of, ofloxacin; Ox, oxacillin; Pi, piperacillin, PiT, piperacillin + tazobactam; T, tetracycline; Tig, tigecycline.

Further, comparison of antimicrobial susceptibility of streptococci from different hosts revealed that streptococci from human clinical samples were more often ($p < 0.0\%$) resistant to Car, Cot, Of, Cfx, and Lin than isolates from sick dogs and domestic animals; to AO, BLO, Tet, CPZ, Ert, Az, and E than isolates from sick domestic animals; and to RWO than those from sick dogs, but more susceptible to amoxicillin + sulbactam (AS) than isolates from ailing dogs (p, 0.04). Streptococci from sick dogs were more often ($p \leq 0.02$) resistant to AS and AZ, and less often to RWO and Tig than those from domestic animals.

The MHDR was highest in streptococci from domestic animals (80%) followed by strains from poultry (72.76%), wild/zoo birds (69.23%), and the least in strains from horses (38.98%), while MDR was detected at highest levels in streptococci from wild and zoo animals (87.8%) followed by poultry birds (82.36%), humans, pigs and domestic animals (~80%), and the least in strains isolated from healthy human beings.

Resistance to macrolides (68.18%) and penicillin (86.36%) was the maximum in streptococci from samples of poultry birds (Table 5). Resistance to vancomycin was the maximum in streptococci from non-clinical samples of domestic animals (87.5%), and resistance to tetracycline was the most common among strain samples of wild and zoo animals (78.05%). Resistance to CNH, Car, TO, GO, Ox, AC, G, C, CTX, CTX-C, CTR, and CR in streptococci of poultry birds origin was significantly higher ($p < 0.05$) than those originating from humans, dogs, domestic animals, and horse clinical samples. Similarly, streptococci isolated from wild and zoo animals were more often ($p < 0.05$) resistant than streptococci from humans, dogs, domestic animals, and horses to G, CTX, CTX-C, CTR, Mox, CFX, CFL, and CR. There was also a significant difference concerning other drugs among strains of different origins. Among all streptococci, strains of equine origin were significantly ($p < 0.05$) more often MDR and MHDR than streptococci from other animals, birds, and humans.

On analysing antimicrobial resistance in different species of streptococci, it was evident that *S. anginosus*, *S. pneumoniae*, and *S. porcinus* isolates were excessively ($p < 0.05$) resistant to ajowan oil than isolates of *S. dysgalactiae* ssp. *equisimilis*, *S. iniae, S. pyogenese*, *S. sanguinis*, *S. suis*, and *S. ubersis* isolates; *S. anginosus* isolates are more resistant to HBO than isolates of *S. agalactiae, S. dysgalactiae*, and *S. pyogenes*; more resistant to citral than *S. agalactiae*, *S. dysgalactiae*, *S. iniae,* and *S. suis* isolates; more resistant to lincomycin than *S. dysgalactiae*, and *S. equi* isolates. Streptococci of different species varied significantly ($p < 0.05$) in their susceptibility to different antibiotics, as *S. agalactiae* were more often resistant to linezolid than isolates of *S. equi*, *S. iniae*, *S. sius*, and *S. uberis,* while more resistant to tigecycline than isolates of *S. anginosus*, *S. pyogenes*, and *S. suis*. Similarly, *S. anginosus* isolates were more often ($p < 0.05$) resistant to AO than isolates belonging to *S. dysgalactiae*, *S. iniae*, *S. sanguinis*, *S. suis* and *S. uberis*, and more resistant to linezolid than isolates of *S. dysgalactiae, S. equi*, and *S. iniae*. *Streptococcus bovis* isolates were more often resistant to guggul oil than isolates of *S. dysgalactiae*, *S. equi*, *S. iniae*, *S. anginosus*, *S. porcinus*, *S. suis*, and *S. uberiss*; and also more often resistant to ertapenem than isolates belonging to *S. agalactiae, S. dysgalactiae*, *S. equi*, *S. iniae*, *S. anginosus*, *S. pyogenes,* and *S. uberis*. Isolates of *S. dysgalactiae* ssp. *dysgalactiae* appeared often more ($p < 0.05$) susceptible to many of the antibiotics than isolates belonging to other species, but they were more often ($p < 0.05$) resistant to nitrofurantoin than *S. iniae* and *S. pyogenes* isolates, to gentamicin and minocycline than *S. pyogenes* and *S. suis* isolates, respectively. Isolates of *S. dysgalactiae* ssp. *equisimilis* were often more resistant to penicillins than isolates of sister subspecies *S. dysgalactiae* ssp. *dysgalactiae*, and also more than isolates of *S. equi, S. pyogenes,* and *S. suis*. Isolates of *S. iniae* were more often susceptible to many of the antibiotics, similar to *S. dysgalactiae* isolates, but were more commonly ($p < 0.05$) resistant to vancomycin than isolates belonging to *S. agalactiae, S. equi, S. pyogenes,* and *S. sanguinis*, and more resistant to clindamycin than isolates of *S. dysgalactiae* ssp. *dysgalactiae, S. pyogenes*, and *S. uberis*. Isolates of *S. pneumoniae* were more often ($p < 0.05$) resistant to Nf than isolates of *S. dysgalactiae* ssp. *equisimilis*, *S. iniae*, and *S. pyogenese* species, and are more resistant to amoxicillin than isolates of *S. dysgalactiae* ssp. *dysgalactiae*, and *S. equi*. Isolates of *S. porcinus* were more often ($p < 0.05$) resistant to HBO than isolates of *S. agalactiae*, *S. dysgalactiae*, and to tetracyclines than isolates of *S. aglactiae*, *S. anginosus*, *S. bovis*, *S. equi*, *S. iniae*, *S. pneumoniae*, *S. pyogenes*, and *S. suis*. *Streptococcus pyogenes* isolates were more commonly ($p < 0.05$) susceptible than many other species strains of streptococci for several antimicrobials, but more often resistant to lincomycin than *S. agalactiae, S. dysgalactiae,* and *S. equi* isolates, more resistant to LGO and citral than *S. agalactiae*, *S. dysgalactiae* ssp. *dysgalactiae*, and *S. suis* isolates. *Streptococcus suis* isolates more frequently resisted ertapenem and other carbapenems than isolates belonging to *S. agalactiae, S. dysgalactiae, S. equi*, *S. iniae*, *S. pyogenes*, and *S. sanguinis*; to vancomycin than isolates of *S. aglactiae*, and *S. pyogenes*. Although *S. uberis* isolates were often susceptible to most of the herbals and antibiotics tested, they were more often ($p < 0.05$) resistant to citral than *S. dysgalactiae* ssp. *dysgalactiae*, *S. iniae*, *S. sanguinis*, and *S. suis* isolates.

Lancefield group D, E, F, G, and non-Lancefield group streptococci were more often ($p < 0.05$) resistant to many of the antimicrobials than isolates belonging to other Lancefield groups. Specifically, more resistant to cotrimoxazole and carbapenems than streptococci of Lancefield groups A, B, and C isolates. The Group C streptococci were also more often susceptible to many herbal antimicrobials like AO, HBO, and LGO than streptococci belonging to Lancefield groups A, D, E, F, G, H, and non-Lancefield group isolates (Table 9).

4. Discussion

In the present study, the most commonly isolated streptococci from clinical samples were *S. pyogenes* (Group A; 140), followed by *S. anginosus* (Group E, F; 111), *S. dysgalactiae* ssp. *equisimilis* (Group C; 34) *S. pneumoniae* (29), *S. equi* ssp. *zooepidemicus* (group C; 17), *S. agalactiae* (Group B; 16), and *S. bovis* (Group D, 11) from samples of a wide spectrum of clinical infections in a range of hosts. The previous studies also documented that the most of the streptococcal infections in humans are attributed to group A (*S. pyogenes*), and Group B streptococci (GBS) primarily infect animals, and they are primarily responsible for UTI, pyelonephritis, low birth weight, and preterm deliveries in infected women and mastitis in animals [22]. *Streptococcus pyogenes* strains are equally pathogenic to humans and animals; they have been reported to be associated with a number of ailments, like abortions and ear infections in animals [23, 24]. The GBS were detected in the present study mostly from sick animals (buffalo, cattle, deer, dogs, and horses) and were also isolated as a cause of UTI in two human cases, as reported earlier [22]. Though, GBS are often considered harmless commensals in humans colonizing the gastrointestinal and genitourinary tracts of up to 30% of healthy human adults, they sometimes causes serious infections like septicaemia [22, 25, 26]. Many GBS strains also infect neonates, probably due to colonization of the recto-vaginal region of pregnant women and acquisition of infection by neonates during delivery [25, 26].

Of the 55 Group C streptococci (GCS) isolates from clinical sources, 20 were from horses suffering from respiratory tract infections, four caused infections in humans (3 skin infections and 1 UTI), and the rest were from a wide range of animals and birds, causing many different types of illnesses. Earlier, GCS were primarily considered important pathogens of equines, causing serious chronic illnesses like strangles and wound infections [27, 28]; they are rarely reported to cause human infections despite being commensal human flora of the nasopharynx, skin, or genital tract [29]. However, recently they have been reported to cause otitis media in dogs [23]. There are reports of their infections similar to group A streptococci in humans and other animals [29]. The observations indicated a strong zoonotic potential of group C streptococci similar to earlier observations [30].

Of the 17 Group D streptococci (GDS), 15 were isolated from animals, mostly from pigs suffering from pneumonia and septicemic infections, and two were from sore throat cases of humans. Similar to GCS, GDS (*S. bovis*, *S. equinus, S. suis*) are primarily known as animal pathogens but in recent past they have been identified as emerging zoonotic pathogens often associated with colorectal carcinoma, inflammatory bowel disease, peptic ulcer disease, diverticular disease, and gastrointestinal bleeding, bacteraemia, endocarditis in humans but have rarely been associated with sore throat infections [31-33].

Streptococcus anginosus group (*S. anginosus*, *S. milleri*, *S. mitis*, *S. mitor*, etc.) streptococci were one of the most frequently detected groups of streptococci in the study, isolated from 122 clinical cases affecting a wide range of hosts and causing many different types of ailments. Of 122 isolates, 29 were from humans suffering from bacteraemia 1, dental infections 3, intestinal abscess 1, upper respiratory tract infections 13, and UTIs 11. *Streptococcus anginosus* group strains are often α-haemolytic [33], have been shown to belong to several Lancefield antigens (A, C, E, F, G), and often cause serious infections like abdominal sepsis, brain abscesses, endocarditis in humans [34-36] and pulmonary infections in animals [37].

In the study, among the group G and H streptococci, *S. canis* was detected from a case of septicemia in a hyena, two cases of UTI in dogs, and a case of pustular dermatitis in a dog. Most of the *S. dysgalactiae* ssp. *dysgalactiae* were isolated from mastitis cases in cows (4) and buffalo (1), and also from a case of abortion in cows. All five *S. intestinalis* strains were detected from milk samples of cow mastitis cases (5), but *S. sanguinis* isolates originated from more diverse cases viz., faeces of diarrhoeic pigs (3), abortion in cows (2), mastitis milk of a cow, and throat swab of a man suffering from chronic sore throat. Streptococci of group G and H (*S. canis*, *S. dysgalactiae ssp. dysgalactiae*, *S. sanguinus*) are reported to be important pathogens of dogs, cats, and cattle but are fast emerging as a zoonotic infection in human medicine [38, 39]. In humans, Group G streptococci are often isolated from the gastrointestinal tract of people with one or other underlying medical disorder, viz., diabetes mellitus, malignancy, alcohol abuse, or immunosuppression [38, 39]. In dogs, *S. canis* causes otitis, skin infections, GIT infections, urogenital infections, rhinitis, septicaemia, pneumonia, placentitis and neonatal death but cats are usually asymptomatic carriers [38, 40], however, it seems to be a first report of isolation of *S. canis* from a septicemic case of hyena. Strains of *S. dysgalactiae* ssp. *dysgalactiae* are often the cause of contagious mastitis in cattle as observed in the present study [41, 42]. Though it is considered a major cause of mastitis only, its isolation from cases of abortions and metritis indicates its widening pathogenic potential. Though it was not detected in any of the human clinical samples in the present study, it is reported as an emerging human pathogen causing serious infections similar to *S. pyogenes* [43, 44]. Strains of *S. sanguinis/S. sanguis* are considered normal oral inhabitants but may accidentally (after some mechanical injury) enter the bloodstream and colonize heart valves, causing sub-acute endocarditis [45, 46]. However, our observations indicated that they had pathogenic significance besides just being commensals or opportunists. Its isolation from a chronic sore throat case indicated its persistent biofilm-forming ability in the oral cavity, and observations are in concurrence with earlier reports of its oral biofilm-forming ability, and its ability to interact with caries- and periodontitis-associated pathogens [47].

Streptococcus porcinus and *S. suis* strains are primarily considered residents of the pig gastrointestinal tract [48]. However, in our study only six of the 36 isolates were from pigs and rest were from other animals (19), and birds (5) suffering from a wide range of ailments viz., septicemic deaths (5 birds), bacteraemia (9), abortion (1), otitis (1), posthitis (1), bronchitis (2), pneumonia (3), UTI (1), wound infections (6) and diarrhoea (1), and six were from humans suffering from UTI (3), bacteremia (1) and sore throat. Our observations are in concurrence with earlier reports [23, 48]. *Streptococcus porcinus* is known for its pathogenicity in pigs, causing infections of lymph nodes, the lower respiratory tract, and the genital tract of pigs, but can infect other animals and humans [48].

Streptococci iniae were isolated from clinical samples associated with abortion in cattle 4, septicemia (1 pig), and three (faeces of a pig and two horses). Though *S. iniae* is primarily considered a pathogen of aquatic animals causing lethal infections in farmed fish, and golf ball disease in dolphins, it has also been identified as an emerging zoonotic pathogen affecting persons in water sports and fish handling [49, 50] but rarely reported causing infections like abortions and septicemia in animals as observed in the present study indicating the widening of host range of this emerging zoonotic pathogen.

Of the six isolates of *S. uberis*, four were from cases of mastitis and one each was from abortion and septicemic cases. It is considered a cause of contagious mastitis but has rarely been reported to cause other infections [41, 42].

In the present study, *S. adjacens* was associated with a case of human UTI, and *S. defactivus* from two cases of abortions in goats, and also as commensals in human urine. These two nutritional variant streptococci are now known as *Granulicatella adjacens* and *Abiotophia defactiva* and often get missed in routine diagnostic laboratories, due to their fastidious nature, may also cause serious life-threatening infections like internal abscesses and endocarditis, but are rarely reported as a cause of UTI and abortions [51-53].

Streptococcus morbilorum (now also known as *Gemella morbilorum*) was from a case of mastitis-metritis syndrome and was also isolated from a deep vaginal swab sample of a cow. It is often considered a commensal in oropharyngeal and gastrointestinal tracts and may rarely cause infective endocarditis, deep visceral abscesses, and soft tissue necrotizing infections [54] has not been reported earlier as a cause of genital tract infection.

Of the four *S. phocae* isolates in the study, one was from tongue scrapings of a persistent human glossitis case, and one each was from a case of mastitis in a buffalo, otitis in a dog, and from the urine of a healthy person. Though it is frequently isolated from dead marine mammals, its pathogenicity is largely obscure [55]. It has rarely been reported to cause infections in humans and terrestrial animals, except for a reported case of dermal infections in mink [56].

Though *S. rattus* is not known to cause any serious illness except causing foul smell from the mouth [57], an isolate of *S. rattus* in the study was from a guinea pig that died of a liver abscess and appears to be the first report of soft tissue abscess caused by *S. rattus*. It is usually isolated from healthy individuals as a component of dental biofilms and is known to produce a bacteriocin-inhibiting cariogenic species of the Mutans group of Streptococcus and is considered a good bacterium [57].

The two isolates of *S. salivaris* were from one sample each of tongue swabs from glossitis and a swab of a sore throat case in a child. Similar to *S. rattus*, *S. salivaris* is also included in a beneficial group of streptococci. It is a common inhabitant of the human oral cavity and rarely reported to cause infections, and it produces bacteriocin to inhibit other potential pathogens, and possesses anti-inflammatory activity [58]. The isolation of *S. salivaris* in the present study from clinical samples might represent a contaminant in the samples, as it is common at the sampling site, the oral cavity [58]. All three isolates of *S. alactolyticus* were from urine of human UTI cases; it belongs to the *S. bovis* complex and has been reported to cause infective endocarditis but has rarely been associated with UTI [31, 59].

In the study, five *S. intestinalis* isolates were from milk samples of cattle mastitis cases. Though *S. intestinalis* is a common inhabitant of the pig colon and isolated from ≥50% of pig faeces [33], it is rarely reported to cause any other infection as observed in the present investigation.

Though *Streptococcus macacae* in the study was isolated from a case of bacteraemia in a rhino, it has rarely been reported to cause any disease and is often present in dental plaques of primates [60].

Although streptococci may cause a wide range of minor to serious life-threatening infections in humans, birds, and animals, the emergence of antimicrobial resistance in streptococci is rarely considered a serious threat. In the present study, 73.41% and 60.32% of streptococci had MDR and multiple drug resistance and were identified are more drug-resistant than other Gram-positive bacteria like *Mammalicoccus* spp. [61] and staphylococci in the region of the study [62]. Among clinical streptococcal isolates, >57% were resistant to one or other tetracycline group antibiotics, >47% to lincomycin, > 36% to macrolides, >44% to aminoglycosides, and >27% to fluoroquinolones. Though there are a few studies on comprehensive drug resistance encompassing streptococci of different species, several studies have reported the emergence of MDR in different species of streptococci viz., of MDR in *S. suis* and *S. porcinus* along with *S. canis* strains is reported as a big threat to swine production [62], emergence of drug resistance in group D streptococci [63, 64], in *S. anginosus* group [35, 59] *S. bovis* complex [31, 59] has become a human health menace.

In *S. anginosus* group appeared as one of the most common group of drug resistant streptococci in Bareilly region with MDR in >79% and MHDR in >2/3rd of the isolates. Though AMR has been reported as an emerging problem in *S. anginosus* group streptococci, infections are reported satisfactorily treatable with penicillin G and cephalosporins [35]. However, in the present study >61% and > 75% of the *S. anginosus* group streptococci isolates were resistant to penicillins and cephalosporins and only imipenem inhibited >80% of the isolates indicating the need to search for alternative therapeutic agents.

Emerging MDR (resistance against tetracyclines, lincosamides, macrolides, and aminoglycosides) in *S. suis* and *S. porcinus* along with *S. canis* is a big threat to swine production [63], however, based the present study doxycycline, minocycline, ofloxacin and cefotaxime with clavulanic acid may be recommended as the drug of choice as they inhibited ≥80% of the isolates.

Further, more than 50% of the isolates of Group D streptococci were resistant to CTX, CTR, Mox, Amx, Azt, CTZ-C, CFX, CTZ, Spt, Cot, Cp, Ert, Cfd and >84% had MDR. The emergence of AMR in group D streptococci has made these pathogens an important health menace [63, 64]. They are reported resistant to levofloxacin, erythromycin, gentamicin, and tetracycline but treatable with erythromycin, clindamycin, cefotaxime, and chloramphenicol [63, 64]. However, in the present study none of these antibiotics were effective to inhibit ≥75% of the isolates and best antibiotics inhibiting >80% of the isolates of this group of streptococci were doxycycline, minocycline, ofloxacin and cefotaxime with clavulanic acid.

Though, >60% of the streptococci (58.82% of clinical origin) isolates tested had multiple herbal drug resistance (MHDR several herbal origin antimicrobial including > 70% of streptococci including carvacrol (93.18%), thyme oil (78.61%), cinnamaldehyde (74.23%), ajowan oil (73.08%) and cinnamon oil (70.50%) inhibited >70 of the streptococci and may be considered an important alternative to treat AMR streptococci. Thyme oil and carvacrol (active ingredient of thyme oil, ajowan oil and oregano oil) inhibited >80% of streptococci of clinical origin. Similar observations indicating cinnamaldehyde and carvacrol as the most effective herbal origin antimicrobials have been reported earlier too [65, 66]. However, the bigger question is how to use the herbals, and more research is required [67] even for using herbal antimicrobials for topical applications. Though observations on the anti-streptococcal activity of herbal antimicrobials are encouraging there is a paucity of earlier observations to discuss further.

5. Strengths and Limitations of the Study

The major limitation of the study was the regionality of the study even not encompassing a country, all isolates were from the Bareilly region and that too from referred cases not representing the true picture of AMR in streptococci of the Bareilly region. However, at the other end, the study had considerable strength in analysing AMR in >500 isolates of streptococci of diverse origin and belonging to more than two dozen of species of clinically important streptococci.

6. Conclusion

The study concluded that there may be several streptococci infecting without respecting different host boundaries indicating their true zoonotic nature. Not only MDR but also MHDR in the majority of the streptococci revealed the real AMR threat in the therapeutic management of streptococcal infections. Given the rising resistance to conventional antibiotics, the findings underscore the need for alternative treatments, including herbal antimicrobials. The study indicated the need for utmost care in antimicrobial therapy of streptococcal infections as many of the globally recommended groups of antibiotics for treating streptococcal infections were inefficacious against the majority of the isolates. The study suggests an antimicrobial susceptibility assay in all cases of streptococcal infections for selection of the suitable and effective antimicrobials for treatment.

Author Contributions

Singh BR conceived the idea, designed the study, implemented the plan, analyzed the study results and drafted the manuscript; Chandra M confirmed cultures using MALDI-TOF MS, Pawde AM, Yadav A, Jayakumar V and Agri H retrieved data and arranged for analysis, Agrawal R, Yadav A, Jayakumar V, Karthikeyan R, Kumar A, and Agri H did bacteriological analysis of samples and vancomycin susceptibility testing, Singh BR, Pawde AM, Kumar A, Yadav A, Jayakumar V and Agri H writing and reviewing of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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