EMERGING DRUG RESISTANT STREPTOCOCCAL ISOLATES FROM SKIN AND SOFT TISSUE INFECTIONS

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ABSTRACT

BACKGROUND

Streptococcal species are the cause of a variety of skin and soft tissue infections (SSTIs) some of which are severe and can be life-threatening. There are currently 74 species under the genus *Streptococcus*. *Streptococcus pyogenes* is the most pathogenic bacterium in the genus *Streptococcus*. We wanted to assess pyogenic potential of different species *Streptococcus* and their drug susceptibility patterns.

METHODS

This is a retrospective descriptive study done from Jan 2017 to Nov 2017. Total enrolled pus samples were 3300. The samples were processed conventionally and antibiotic sensitivity testing (AST) of all isolates was performed by Kirby Bauer's disc diffusion method as per CLSI guidelines.

RESULTS

4.63 % were culture positive for streptococcal isolates. Among streptococcal isolates, 128/153 (83.66%) were *Enterococcus* species, 12/153 (7.84%) were *Streptococcus pyogenes* and 10/153 (6.53%) were *Streptococcus agalactiae* and 3/153 (1.96%) were *Streptococcus pneumoniae*. For all Streptococcal isolates (153), 64% (102/160) were male patients and 36% (58/160) were female patients. Drug resistance is increasing for all the streptococcal species, and resistance to even high-end drugs like vancomycin was seen to be around 7.5% in *Enterococcus* spp. Almost one third of the isolates of *Streptococcus pyogenes* were resistant to Erythromycin and Gentamicin. No resistance was seen to Linezolid in any of the Streptococcal isolates.

CONCLUSIONS

Streptococcus species such as *S. pyogenes*, *S. agalactiae* and *Streptococcus pneumoniae* are seen to be increasing in SSTIs. Bacterial isolates exhibited high to moderate levels of resistance against different classes of antibiotics. A continuous inspection should be carried out to monitor the susceptibility of these pathogens and chose appropriate regimens both for prophylaxis and treatment of surgical wound infections. There is a need to create a viable antibiotic policy and draft guidelines to prevent or reduce undirected use of antibiotics, and conserve their effectiveness for better patient management. Consistent dialogue between the microbiology department and the surgeons is required for management of such cases.

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BACKGROUND

Streptococcal species are the cause of a variety of skin and soft tissue infections (SSTIs) some of which are severe and can be life-threatening. There are currently 74 species under the genus *Streptococcus*.¹ *Streptococcus pyogenes* the most pathogenic bacterium in the genus *Streptococcus*; the agent that causes bacterial pharyngitis, impetigo, scarlet fever, rheumatic fever and a host of other infections including severe invasive diseases.² It is implicated in a wide variety of SSTIs including impetigo, erysipelas, cellulitis, necrotizing fasciitis, myositis and myonecrosis.³ The past two decades

have also witnessed a resurgence of several infective syndromes of *S. pyogenes*, most notably necrotizing skin and soft tissue infections and scarlet fever.⁴ *Streptococcus agalactiae* is a gram-positive coccus belonging to group B of the Lancefield classification.⁵

Today, Group B *Streptococcus* (GBS) colonizes gastrointestinal tract and genitourinary tract of about 30% of healthy individuals, without any symptoms.^{6,7} During the past three decades there have been reports of increasingly invasive infections in adults unrelated to pregnancy, although arthritis and osteomyelitis were still considered rare.⁸ A recent multi-centre study of 171 patients with invasive GBS infections in non-pregnant adults has reported SSTIs in 31% of cases.⁹

Streptococcus pneumoniae commonly and asymptotically colonizes the upper respiratory tract of children and is a major cause of respiratory and meningeal infections. SSTIs constitute around 2.2% of all pneumococcal infections where a breakdown of the normal mucosal barrier plays a major role in the development of bacteraemia and other soft tissue infection.¹⁰ Besides, haemolytic streptococci, *Enterococcus* spp. are being increasingly isolated from SSTIs.¹¹ Enterococci are generally known to cause urinary,

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bloodstream and gastrointestinal infections. However, the increasing use of cephalosporins has contributed to emergence of Enterococci as etiological agents of SSTIs especially after trauma and surgery.¹²

The inadvertent use of antibiotics leading to emergence of drug resistant pathogenesis a great healthcare challenge. Although Penicillin remains the drug of choice for Group A and Group B Streptococci, resistance to macrolides, lincosamides and fluoroquinolones has emerged.¹³ Similarly, incidence of difficult to treat Vancomycin resistant Enterococci (VRE) too is on the rise.¹⁴

Thus, the present study was aimed to assess pyogenic potential of different species of Streptococcus and their drug susceptibility patterns from patients with pus and/or wound discharge.

METHODS

Study Design

This is a retrospective descriptive study conducted at the department of microbiology in a tertiary care hospital of northern India from January 2017 to November 2017.

Sampling Methods

Microbiology database was screened for all Streptococcal isolates from pus, wound discharge and tissue samples collected from patients with SSTIs. A total number of 3300 pus samples were obtained for aerobic culture and sensitivity from different In-patient department (IPD) & Outpatient department (OPD) of our hospital. Pus samples were collected with sterile disposable cotton swabs and aspirates in syringe and were transported and processed in the microbiology laboratory immediately.

Sample Processing

They were inoculated on to 5% sheep Blood agar (BA), MacConkey agar (MA) and Brain heart infusion broth. Direct microscopy was performed by gram stain examination of the samples. All available clinical details of patients were noted. Culture plates were incubated at 37°C for 24 to 48 hrs. under aerobic condition. After incubation, identification of bacteria from positive cultures was done using standard phenotypic methods. All Streptococcal isolates were confirmed by conventional and serological tests. The antibiotic sensitivity testing (AST) of all isolates was performed by disk diffusion method on Muller Hinton agar and interpreted as per CLSI guidelines and classified as sensitive and resistant (CLSI 2017).^[15] Antimicrobial susceptibility testing was performed against the following antibiotics: ampicillin (30 µg), gentamicin (10 µg and 120 µg), cefoxitin (30 µg), linezolid (30 µg), vancomycin (30 µg), ciprofloxacin (5 µg), erythromycin (10 µg), clindamycin (2 µg), Trimethoprim/Sulfamethoxazole (1.25 µg/23.75 µg). Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 were used for quality control. All the culture media, biochemical media and antibiotics used were obtained from Hi Media.

Statistical Analysis

Results obtained were entered in the excel sheet and analyzed by using MS Excel, 2007 version. The percentage of sensitivity and resistance was calculated for all the organisms.

RESULTS

A total of 3300 samples were received during the study period from January 2017 to November 2017, out of which 153 (4.63%) were culture positive for streptococcal isolates. Among streptococcal isolates 128/153 (83.66%) were Enterococcus species, 12/153 (7.84%) were Streptococcus pyogenes, 10/153 (6.53%) were Streptococcus agalactiae and 3/153 (1.96%) were Streptococcus pneumoniae.

Antibiotic	% R	%IS	%S
Ampicillin	63.4	00	36.6
Gentamicin High Level (120)	44.3	00	55.7
Erythromycin	70.3	14.1	15.6
Ciprofloxacin	46	24	30
Linezolid	00	00	100
Vancomycin	7.5	00	92.5
Chloramphenicol	16	4.5	79.5

Table 1. Antimicrobial Profile of Enterococcus Species (n=128)

Antibiotic Name	% R	% IS	% S
Ampicillin	00	00	100
Gentamicin	33.33	00	66.67
Clindamycin	00	00	100
Erythromycin	33.33	00	66.67
Linezolid	00	00	100
Vancomycin	00	00	100

Table 2. Antimicrobial Profile of Streptococcus Pyogenes (n=12)

Antibiotic Name	% R	% IS	% S
Penicillin	00	00	100
Ampicillin	00	00	100
Cefoxitin	20	20	60
Gentamicin	40	20	40
Clindamycin	30	00	70
Erythromycin	20	10	70
Linezolid	00	00	100
Vancomycin	00	00	100
Chloramphenicol	40	10	50
Tetracycline	100	00	00

Table 3. Antimicrobial Profile of Streptococcus Agalactiae (n=10)

In 128 isolates of Enterococcus species 60% were from In-patients ward (IPD) and 40% were from outpatient department (OPD). For Streptococcus pyogenes, Streptococcus pneumoniae and Streptococcus agalactiae 90% were from IPD and 10% were from OPD. The most common specialities were General Surgery followed by Orthopaedics for all kinds of isolates. For all Streptococcal isolates (153), 64% (98/153) were male patients and 36% (55/153) were female patients. The level of resistance was very high in Enterococcus species as is shown in Table 1. Almost 7.5% of the isolates were resistant to high end drugs like Vancomycin. Even in Streptococcus pyogenes resistance to commonly used antimicrobials such as Erythromycin and Gentamicin was high (up to 33.33%) (Table 2). The Antimicrobial susceptibility of Streptococcus agalactiae is shown in Table 3. In case of Streptococcus pneumoniae it is difficult to comment on the level of resistance as the number of isolates was less but out of three isolates seen in our study one isolate was resistant to Erythromycin.

DISCUSSION

Skin and soft tissue infections are one of the most common infectious diseases worldwide.¹⁶ The treatment of serious SSTIs require prompt anticipation of the severity of infection and timely debridement along with antibiotic cover for the control of infection. Though methicillin resistant *Staphylococcus aureus* (MRSA) remains one of the most common pathogens responsible for SSTIs but the etiological spectrum is changing, and now multidrug resistant Gram-negative organisms and vancomycin resistant enterococcus are amongst few emerging organisms from SSTIs. In the present study 4.63% of the samples were culture positive for streptococcal isolates. The most common was *Enterococcus* species (83.66%) followed by *Streptococcus pyogenes* (7.84%), *Streptococcus agalactiae* (6.53%) and *Streptococcus pneumoniae* (1.96%). The drug resistant *Enterococcus* species has emerged as an important nosocomial pathogen over past few years. In our study, 63.4% of Enterococcal isolates were resistant to Ampicillin while 70.3% showed resistance against Erythromycin, 44.3% were resistant against high-level Gentamicin (HLG). Vancomycin resistant Enterococcal isolates were 7.5%. No resistance was found against linezolid. Such high level of drug resistance to multiple antibiotics in Enterococcal isolates have been observed by other authors also.^{17,18} Recent studies have shown that the vancomycin resistance can vary between 1.7-20% in the tertiary care hospitals of India as was seen in the present study also.¹⁹⁻²¹

In this present study, we have also investigated antimicrobial activity against *S. pyogenes* isolates. Resistance was seen to the commonly used antimicrobials such as Erythromycin, Gentamicin (33.3%). No resistance was seen against linezolid and vancomycin. Takashi Takahashi et al has recently reported a high frequency of resistance to erythromycin in *Streptococcus pyogenes*, particularly in countries where antibiotics are overused.²² Tamayo et al reported the erythromycin resistance rate to be 21.7% in the study done in Spain in 2004.²³ Alberti et al had also reported a very high level of resistance to fluoroquinolones to the tune of 63.3% amongst *S. pyogenes* isolates.²⁴ The increase in resistance in *Streptococcus pyogenes* isolates is a cause of worry as the SSTI caused by it can progress rapidly and may lead to necrotizing fasciitis and other sequelae if not treated promptly. This becomes important in patients who are allergic to penicillins where these alternate drugs would need to be used.

Streptococcus agalactiae is a major pathogen in neonatal infections. However, an increasing number of diseases caused by GBS in non-pregnant adult-invasive strains has been observed.^{25, 26} In adults, *S. agalactiae* causes skin and soft tissue infections, bacteraemia, urinary tract infections, pneumonia, osteomyelitis, meningitis, endocarditis, and streptococcal toxic shock syndrome.^{25, 26} Risk factors associated with invasive GBS in adults are old age, diabetes mellitus, neurologic diseases, cirrhosis or other liver diseases, stroke, breast cancer, and renal failure.²⁷⁻²⁹ In our study, ten isolates of *Streptococcus agalactiae* and three isolates of *Streptococcus pneumoniae* were recovered; considered as rare pathogens in suppurative infection. The increase in the

prevalence of these relatively uncommon isolates and rising prevalence of drug resistance from the SSTIs warrants that culture and sensitivity should be done from all the suspected cases.

CONCLUSIONS

Streptococcus species such as *S. pyogenes*, *S. Agalactiae* and *Streptococcus pneumoniae* are seen to be increasing in SSTIs. Bacterial isolates exhibited high to moderate levels of resistance against different classes of antibiotics. The susceptibility data from this report may be worth consideration while implementing empiric treatment strategies for pyogenic infections. A continuous inspection should be carried out to monitor the susceptibility of these pathogens and chose appropriate regimens both for prophylaxis and treatment of surgical wound infections. There is a need to create a viable antibiotic policy and draft guidelines to prevent or reduce undirected use of antibiotics, and conserve their effectiveness for better patient management. Consistent dialogue between the microbiology department and the surgeons is required for management of such cases.

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