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Spectrum of beta haemolytic infections in ENT patients

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Abstract

Objectives of the study are to find out the incidence of beta-haemolytic bacterial infection of throat, nose, and ear, to identify various beta-haemolytic isolates and to study antibiotic susceptibility pattern of various beta haemolytic isolates. A total of 53 symptomatic patients attended ENT OPD among which 12 were children and 41 were adults. A total of 35 throat swabs, 5 nasal swabs and 13 ear swabs were collected from the patients with symptoms of pharyngitis or nasal discharge or ear discharge. Out of 53 samples, culture for β-haemolysis was positive in 23 samples. Out of 53 samples, culture was positive in 23 samples with throat swab positive in 13 samples, ear swab positive in 8 samples and nasal swab positive in 2 samples. Twenty strains of *Staphylococcus aureus*, 2 strains of *Pseudomonas aeruginosa* and 1 strain of *Streptococcus pyogenes* were isolated. The isolation rate of *S. aureus* was found to be statistically significant when compared between the isolation rate of *S.aureus* and *P. aeruginosa* and isolation rate of *S.aureus* and *S. pyogens* . Four isolates of *S. aureus* were sensitive to penicillin, 2 isolates were moderately sensitive and fourteen isolates were resistant. Eleven strains of *S. aureus* were sensitive to methicillin. Methicillin resistant was seen in 9 strains of S. aureus. The single strain of *S. pyogenes* isolated was either moderately sensitive or resistant to the used antibiotics, it was not sensitive to any of the used antibiotics. It was moderately sensitive to amoxiclave, clarithromycin, erythromycin and resistant to clindamycin and cefuroxime. 100% strains of *P. aeruginosa* were resistant to ciprofloxacin, gentamicin, piperacillin, ticarcillin, tobramycin and the strains were sensitive only to imipenem (100%).

Keywords: Beta-haemolysis, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, Antibiotic susceptibility test.

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Introduction

Infections of ear, nose and throat (ENT) have a tremendous impact on public health. It is one of the reasons for the patients to visit the primary care providers. Upper respiratory tract infection is caused by either viruses or bacteria and bacterial infection may be primary or secondary to viral infection [1]. Beta-haemolytic bacteria are those which produce beta-haemolysis on blood agar [2]. The common beta-haemolytic bacteria isolated from patients attending ENT Department are *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. This organism are

localised in the oropharynx, nasopharynx, nasal cavity and middle ear causing sore throat, nasal discharge, ear discharge and often fever. The primary pathogen of oropharynx is *S.pyogenes* where *S.aureus* is a secondary pathogen [2]. The highest rate of incidence in ENT infection is chronic suppurative otitis media followed by sinusitis, tonsillitis, pharyngitis and acute suppurative otitis media [3].

The morbidity and mortality associated with otitis media is really a challenge for health care system as there are very few studies done in India to know the burden of the disease in the society, even though it is most prevalent in developing countries like India. The highest prevalence rate for otitis media worldwide is in British Columbia Canada with 15.5%. In India, it is 14.65% in Lucknow and 7.43% in Vellore [4]. The prevalence of beta haemolytic streptococcal sore throat was 13.6% in a rural area in Varanasi, India whereas in Europe in 1984 it was estimated that the prevalence rate was 7.2% [6]. The sensitivity pattern of most of the beta haemolytic organisms show increasingly more resistant to the common and routine antibiotics used in ENT department [7, 8]. The antibiotic susceptibility pattern(AST) for *S.aureus* and *P.aeruginosa* are ampicillin, amikacin, cloxacillin, cephalexin, cefuroxime, chloramphenicol, gatifloxacin, gentamicin, ofloxacin, tobramycin, norfloxacin, levofloxacin and netilimicin[9].The prevalence of antibiotic resistant Group A Streptococci has emerged rapidly in northern India.

In the present study, the bacterial load of beta haemolytic infections as well as their susceptibility pattern in patients suffering from ENT infections in Sikkim population is studied.

Material and methods

The study was conducted in the Department of Microbiology, Sikkim Manipal Institute of Medical Sciences, Gangtok. The study population included the patients who visited ENT OPD of Central Referral Hospital with signs and symptoms of pharyngitis, nasal discharge and ear discharge. A total of 38 throat swabs, 5 nasal swabs and 13 ear swabs were collected from symptomatic cases.

Specimen collection: The throat swab, nasal swab and aural swab were collected by sterile cotton swab. The swabs were immediately transported in sterile cotton plugged test tube to the laboratory.

Processing of the specimen

Direct microscopy: It was done by Gram's method and smears were examined for the type and number of bacteria, pus cells and relationship of bacteria to pus cells.

Culture and sensitivity: Throat swab were inoculated on blood agar plates whereas nasal and ear swabs were inoculated on blood agar and MacConkey agar plates. The plates were incubated in a candle jar (5-10% of carbon dioxide) where as MacConkey agar plates were incubated in ambient air at 37°C for overnight. The plates were examined for the growth of bacteria and the pathogenic colonies were identified by conventional methods. Beta haemolysis was studied on blood agar plates.

Antibiotic susceptibility testing: Antibiotic susceptibility test was performed by Kirby Bauer disc diffusion method. Commercially procured antibiotic disc(Hi Media) used for S.aureus were: cefotaxime(30µg), ciprofloxacin(30µg), cotrimoxazole(25µg), erythromycin(15µg), gentamicin(10µg), oxacillin(1µg) and penicillin(10µg) .

The antibiotic discs used for *P.aeruginosa* were: ciprofloxacin(30µg), gentamycin(10µg), imipenem(10µg), piperacillin(100µg), ticarcillin(75µg) and tobramycin(10µg).

The antibiotics used for *S.pyogenes* were: amoxicillin(20µg), cefuroxime(30µg), clarithromycin(15µg), clindamycin(2µg), erythromycin(15µg) and penicillin(10µg).

Statistical analysis: The difference in proportions was tested for statistical significance using chi square. A p value of <0.05 was considered to be statistically significant.

Results

A total of 35 throat swabs, 5 nasal swabs and 13 ear swabs were collected from the patients attending ENT OPD CRH with symptoms of pharyngitis or nasal discharge or ear discharge. Out of 53 samples, culture was positive in 23 (43.40%) samples with throat swab positive in13 samples (56.52%), ear swab positive in 8 samples (26.09%) and nasal swab positive in 2 samples (8.70%). Table 1 shows β-haemolytic bacteria isolated from ENT OPD

Table - 1. Shows β- haemolytic bacteria isolated from ENT OPD

S.no.	Specimen	Name of organisms	Children			Adults		
			M	F	Total	M	F	**Total**
1.	Throat swab	*S.aureus*	0	2	3	6	4	**10**
		S.pyogenes	1	0		0	0	
		P.aeruginosa	0	0		0	0	
2.	Ear	*S.aureus*	1	1	2	1	3	**6**
		S.pyogenes	0	0		0	0	
		P.aeruginosa	0	0		2	0	
3.	Nose	*S.aureus*	0	2	2	0	0	**6**
		S.pyogenes	0	0		0	0	
		P.aeruginosa	**0**	**0**		**0**	**0**	

Twenty strains of *S. aureus*(37.7%), 2 strains of *P. aeruginosa* (3.77%) and 1 strain of *S. pyogens* (1.89%) were isolated. Among 20 strains of *S. aureus*, 12 (60%) strains were isolated from throat swabs, 6 (30%) were isolated from ear swabs and 2 (10%) were isolated from nasal swabs. Two strains of *P.aeruginosa* (25%) were isolated out of 8 culture positive ear swabs. One strain of *S. pyogens* (7.69%) was isolated out of 13 culture positive throat swabs. *S.aureus* constituted 92.31% (n=12) of culture positive throat swabs and 100% (n=2) of culture positive nasal swabs.

In children throat swab culture was positive in 3 cases (60%), whereas in adult it was positive in 10 cases (33.33%). Ear swab culture was positive in 2 cases (66.67%) in children and 6 cases (60%) in adult. Whereas nasal swab culture was positive in 2 cases (50%) in children and positive nasal swab culture was not seen in adult. Culture positive was seen in 11 male patients (39.29%) and 12 female patients (48%) with throat, ear and nasal swab culture positive in 31.82% (n=7), 80% (n=4) and 0% (n=0) in children and 46.15% (n=6), 50% (n=4) and 50% (n=2) in adults respectively.

Four isolates (20%) of *S. aureus* were sensitive to penicillin, 2 isolates (10%) were moderately sensitive and fourteen isolates (70%) were resistant. Eleven strains (55%) of *S. aureus* were sensitive to methicillin. Methecillin resistant was seen in 9 strains (45%) of *S.aureus.* The strains resistant to methicillin were also resistant to penicillin. Four strains (44.44%) of MRSA also showed resistant to cotrimoxazole, cephotaxime, ciprofloxacin, erythromycin and gentamicin. Remaining 5 strains (55.56%) of MRSA showed resistant to one of the above mentioned antibiotics.

Table – 2. Shows the antibiotic susceptibility pattern for S.aureus.

S. No.	Antibiotics	Susceptibility pattern		
		Sensitive(%)	Moderately sensitive(%)	Resistant(%)
1.	Cephotaxime	13(65)	1(5)	6(30)
2.	Ciprofloxacin	11(55)	1(5)	8(40)
3.	Cotrimoxazole	13(65)	0(0)	7(35)
4.	Erythromycin	1(5)	0(0)	19(95)
5.	Gentamycin	12(60)	1(5)	7(35)
6.	Oxacillin	11(55)	0(0)	9(45)
7.	Penicillin	4(20)	2(10)	14(70)

Table – 3. Shows the antibiotic susceptibility pattern for *S.pyogenes*.

Sl.No.	Antibiotics	Susceptibility pattern		
		Sensitive(%)	Moderately sensitive(%)	Resistant(%)
1.	Amoxiclav	0(0)	1(100)	0 (0)
2.	Clarythromycin	0(0)	1(100)	0 (0)
3.	Clindamycin	0(0)	0 (0)	1(100)
4.	Cefuroxime	0(0)	0 (0)	1(100)
5.	Erythromycin	0(0)	1(100)	0 (0)
6.	Penicillin	0(0)	0 (0)	1 (0)

The only one strain of *S. pyogens* that was isolated, was moderately sensitive to amoxiclave, clarythromycin, erythromycin and was resistant to clindamycin, cefuroxime and penicillin.

Table - 4. Shows the antibiotic susceptibility pattern for *P.aeruginosa*.

Sl.No.	Antibiotics	Susceptibility pattern		
		Sensitive(%)	Moderately sensitive(%)	Resistant(%)
1.	Ciprofloxacin	0(0)	0(0)	2(100)
2.	Gentamicin	0(0)	0(0)	2(100)
3.	Imipenem	2(100)	0(0)	0(0)
4.	Piperacillin	0(0)	0(0)	2(100)
5.	Ticarcillin	0(0)	0(0)	2(100)
6.	Tobramycin	0(0)	0(0)	2(100)

Table 4 shows the antibiotic susceptibility pattern for *P.aeruginosa*. Both the strains of *P.aeruginosa* (100%) were resistant to ciprofloxacin, gentamycin, piperacillin,

ticarcillin, tobramycin and were sensitive only to imipenam.

Discussion

A total of 53 symptomatic patients attended ENT OPD among which 12 (22.64%) were children and 41 (77.36%) were adults. The male female ratio was 1:0.89.Thirty five (66.03%) throat swabs, 13 ear swabs (24.53%) and 5 nasal swabs (9.43%) were collected for culture/sensitivity. Symptomatic throat and ear infections were seen more in adults (85.71%, 76.92% respectively) and it was found to be statistically significant (X^2=104; P=< 0.001 and X^2=58.3; p=<0.001 respectively) whereas symptomatic nasal infection was more common in children (80%) and it was also found to be statistically significant (X^2=72.0; P=< 0.001). Out of 53 samples, culture for β hemolytic bacteria was positive in 23 (43.40%) samples. Twenty strains of *S. aureus* (37.7%), 2 strains of *P. aeruginosa* (3.77%) and 1 strain of *S. pyogens* (1.89%) were isolated. The isolation rate of *S. aureus* was found to be statistically significant when compared between the isolation rate of *S.aureus* and *P. aeruginosa* (X^2 = 18.6; p= < 0.001) and isolation rate of *S.aureus* and *S. pyogens* (X^2=21.4;p=<0.001). Among 20 strains of *S. aureus*, 12 (60%) strains were isolated from throat swabs, 6 (30%) were isolated from ear swabs and 2 (10%) were isolated from nasal swabs. Two strains of *P. aeruginosa* (25%) were isolated out of 8 culture positive ear swabs. One strain of *S. pyogens* (7.69%) was isolated out of 13 culture positive throat swabs. *S. aureus* constituted 92.31% (n=12) of culture positive throat swabs, 100% (n=2) of culture positive nasal swabs and 75% (n=6) of culture positive ear swabs.

In children throat swab culture was positive in 3 cases (60%), whereas in adult it was positive in 10 cases (33.33%). Ear swab culture was positive in 2 cases (66.67%) in children and 6 cases (60%) in adult. Whereas nasal swab culture was positive in 2 cases (50%) in children and positive nasal swab culture was not seen in adult. Culture positive was seen in 11 male patients (39.29%) and 12 female patients (48%) with throat, ear and nasal swab culture positive in 31.82% (n=7), 80% (n=4) and 0% (n=0) of male and 46.15% (n=6), 50% (n=4) and 50% (n=2) of female patients respectively. Sobhan Nandi et al reported no significant difference in the incidence of sore throat as well as *Group A streptococcal* sore throat among males and females [10]. Our study also shows no significant difference in throat swab positive culture between male and female (X^2 = 0.719, P = 0.396). Similar to P.K. Majal et al we also observed no difference between males and females in positive ear swab culture [11]. E.S. Donkar at el reported no significant difference in nasal bacterial colonization between males and females [12]. Whereas we reported positive nasal swab culture only in female children.

Sobhan Nandi et al showed that the prevalence of beta haemolytic streptococcal sore throat was 13.6% in rural area of Varanasi, India [10]. Whereas in our study we found *S. aureus* (92.30%) to be the most common cause of pharyngitis followed by *S. pyogenes* (7.69%) and it was statistically significant (X^2 = 141, P< 0.001). Similar to our study, P.T. Wakodel et al also reported *S.aerues* (25.25%) to be the predominant pathogenic organism in throat followed by *S. pyogenes* (1.05%) [13].

Asif Alam et al found that among 150 pus samples collected from the discharging ear, 82% cases yielded pure culture among which *P. aeruginosa* (52.5%) was the commonest isolate followed by S. aureus(15%) [14]. Whereas the study done by Ekpo et al had shown that *Streptococcus spp* (14.92%) was the most common organism [15]. Contrary to the above findings we observed *S. aureus* (75%) to be the predominant organism followed by *P. aeruginosa*(25%) and it was statistically significant (X^2 = *50.0; p= <0.001).*

E.S. Donkar et al showed that nasal swab culture yielded the growth of S.aureus(49%), coagulase negative staphylococcus(27.4%), S.pneumoniae(4%), S.viridan(4%), S.pyogenes(2%), Klebsiella spp(7%), E.coli(2%), enterobacter spp(2%), Pseudomonas spp(2%) and Morganella morgani(1%) [14]. But in our study nasal swab culture yielded only the growth of S. aureus.

Four isolates (20%) of *S. aureus* were sensitive to penicillin, 2 isolates (10%) were moderately sensitive and fourteen isolates (70%) were resistant. Eleven strains (55%) of *S. aureus* were sensitive to methicillin. Methicillin resistant was seen in 9 strains (45%) of S. aureus. The strains resistant to methicillin were also resistant to penicillin. Four strains (44.44%) of MRSA also showed resistant to cotrimoxazole, cephotaxime, ciprofloxacin, erythromycin and gentamicin. Remaining 5 strains (55.56%) of MRSA showed resistant to one of the above mentioned antibiotics. Majority of the strains of *S. aureus* were sensitive to cephotaxime, cotrimoxazole and gentamicin and it was found to be statistically significant(X^2=18.0, p<0.001, X^2=18.0, p=<0.001 and X^2=8.00,p=0.005 respectively); and the strains of *S.aureus* resistant to erythromycin and penicillin were also found to be statistically significant when compared between sensitive veres moderately sensitive and resistant pattern. Lahari Saikia et al had shown that the maximum isolation of MRSA from ENT infections was found to be resistant to penicillin and cephalexin. They also found that MRSA were susceptible to cotrimoxazole(3.12%), gentamicin(12.5%), erythromycin(18.75%) and clindamycin(43.75%) [16].

Kaplan et al showed that all strains of *S. pyogens* were sensitive to penicillin, clindamycin, ceftriazone but were resistant to erythromycin and azithromycin [17]. Whereas in our study, the single strain of *S. pyogens* isolated was either moderately sensitive or resistant to the used antibiotics, it was not sensitive to any of the used antibiotics.It was moderately sensitive to amoxiclave, clarithromycin, erythromycin and resistant to clindamycin and cefuroxime.

Arshad et al found that majority of organisms isolated from ear infection were resistant to clotimoxazole, ampicillin and erythromycin and 100% strains were sensitive to imipenem and 92% to ciprofloxacin and ofloxacin [18]. Whereas in our study 100% strains of *P. aeruginosa* were resistant to ciprofloxacin, gentamicin, piperacillin, ticarcillin, tobramycin and the strains were sensitive only to imipenem (100%). Out of 6 strains of *S. aureus* isolated from ear infection, 5 strains (83.33%) were resistant to penicillin and 2 strains (33.33%) were resistant to methecillin. Out of 2 MRSA strain, 1(50%) was resistant to cotrimoxazole, cephotaxime, ciprofloxacin, erythromycin and gentamicin. Among non-MRSA strains isolated from ear infection 50% strains were sensitive to penicillin and ≥ strains were sensitive to cotrimoxazole, cephotaxime and gentamicin.

Pant and Rai reported *S.aureus* strains isolated from nose were resistant to ampicillin(38.1%), erythromycin(33.3%), cloxacillin(14.3%), gentamicin(9.5%) and methicillin(9.5%) [19].Our study showed 50% of S.aureus was sentitive to methicillin and 50% strains were MRSA. MSSA strain was sensitive to the used antibiotics except penicillin, erythromycin and ciprofloxacin whereas MRSA strain was resistant to all the antibiotics used for *S. aureus*.

Conclusion

A total of 53 symptomatic patients attended ENT OPD during the study period, among which 12 were children and 41 were adults. The male female ratio was 1:0.89.Thirty five throat swabs, 13 ear swabs and 5 nasal swabs were collected for culture/sensitivity. Symptomatic throat and ear infections were seen more in adults and it was found to be statistically significant whereas symptomatic nasal infection was more common in children and it was also found to be statistically significant. Out of 53 samples, culture for β haemolytic bacteria was positive in 23 samples. Our study showed a high rate of monomicrobial infection. Twenty strains of *S. aureus*, 2 strains of *P. aeruginosa* and 1 strain of *S. pyogenes* were isolated.

In children throat swab culture was positive in 3 cases whereas in adult it was positive in 10 cases. Ear swab culture was positive in 2 cases in children and 6 cases in adult. Whereas nasal swab culture was positive in 2 cases in children and positive nasal swab culture was not seen in adult.

Culture positive was seen in 11 male patients and 12 female patients with throat, ear and nasal swab culture positive in 31.82%, 80% and 0% of male and 46.15%, 50% and 50% of female patients respectively.

Four isolates of *S. aureus* were sensitive to penicillin, 2 isolates were moderately sensitive and fourteen isolates were resistant. Eleven strains of *S. aureus* were sensitive to methicillin. Methecillin resistant was seen in 9 strains of *S. aureus*. The strains resistant to methicillin were also resistant to penicillin. Four strains of MRSA also showed resistant to cotrimoxazole, cephotaxime, ciprofloxacin, erythromycin and gentamicin. Remaining 5 strains of MRSA showed resistant to one of the above mentioned antibiotics. Majority of the strains of *S. aureus* were sensitive to cephotaxime, cotrimoxazole and gentamicin and it was found to be statistically significant and the strains of *S.aureus* resistant to erythromycin and penicillin were also found to be statistically significant when compared between sensitive veres moderately sensitive and resistant pattern.

Further, the single strain of *S. pyogenes* isolated was either moderately sensitive or resistant to the used antibiotics, it was not sensitive to any of the used antibiotics. It was moderately sensitive to amoxiclave, clarithromycin, erythromycin and resistant to clindamycin and cefuroxime. 100% strains of *P.aeruginosa* were resistant to ciprofloxacin, gentamicin, piperacillin, ticarcillin, tobramycin and the strains were sensitive only to imipenem (100%). Out of 6 strains of *S. aureus* isolated from ear infection, 5 strains were resistant to penicillin and 2 strains were resistant to methicillin. Out of 2 MRSA strain, 1 (50%) was resistant to cotrimoxazole, cephotaxim, ciprofloxacin, erythromycin and gentamicin. Among non- MRSA strains isolated from ear infection 50% strains were sensitive to penicillin and ≥ strains were sensitive to cotrimoxazole, cephotaxime and gentamicin.

The present study showed 50% of *S.aureus* was sensitive to methicillin and 50% strains were MRSA. MSSA strain was sensitive to the used antibiotics except penicillin, erythromycin and ciprofloxacin whereas MRSA strain was resistant to all the antibiotics used for *S. aureus.*

The control of ENT infections demands the availability of primary care and appropriate treatment. Guidelines can be formulated for the common ENT infections and followed universally which would promote rational use of antimicrobial agents. Reviewing of microbial sensitivity pattern in all patients at varying time interval in a particular location would give feedback on the use of antimicrobials and would help to formulate the hospital guidelines for rational use of the antibiotics. So an appropriate antimicrobial therapy should be prescribed so that the disease process can be reversed and thereby prevents the longterm sequelae.

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