

## Isolation and Antimicrobial Susceptible Pattern of Bacterial Pathogens from Ear, Nose and Throat of Paediatric Patients

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### ABSTRACT

**Aim:** To investigate the bacterial pathogens of ear, nose and throat (ENT). The aim of the study was to determine the frequency and sensitivity pattern of ENT infections in paediatric patients.

**Methods:** This cross sectional observational study was conducted during July 2012 to December 2012 at The Children's Hospital and Institute of Child Health, Lahore. A total number of 217 samples (172 from ear, 11 from nose and 34 from throat) were collected and processed for microbiological investigations. The organisms were identified by routine microbiological techniques. Antimicrobial susceptibility profile was determined by modified Kirby Bauer disc diffusion method.

**Results:** Out of 217 samples, pathogenic microorganisms were isolated in 136 samples (62.67%) whereas 81(37.33%) samples were culture negative. Single organism type was isolated in 121 samples and 15 samples yielded growth of more than one organism. *Pseudomonas* 53(34.87%) was most frequently isolated organism followed by the *Staphylococcus aureus* 34(22.37%). All of the Gram positive bacteria were susceptible to vancomycin. Majority of Gram negative bacteria were susceptible to sulbactam/cefoperazone (96.46%), piperacillin/tazobactam (96.1%), meropenem (93.81%) and amikacin (84.96%).

**Conclusion:** This study showed that the *Pseudomonas* species, *Staphylococcus aureus* and *Klebsiella* were the primary pathogens of ENT paediatric patients.

**Keywords:** Ear, nose and throat, pathogenic bacteria, paediatric ENT infections

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### INTRODUCTION

Diseases of ear, nose and throat (ENT) affect the functioning of adults as well as children resulting in hearing impairment and hearing loss, learning disability and emotional stress often with significant impairment of the daily life of affected patients. With increase in global population, these infections remain the most important causes of diseases involving upper respiratory tract particularly in children<sup>1</sup>. Upper respiratory tract infections including nasopharyngitis, pharyngitis, tonsillitis and otitis media (OM) constitute 87.5% of the total episodes of respiratory infections.<sup>2</sup> Anatomically, ENT are close to the brain and the nose is also closely related to the orbit, so delayed treatment of infections of the nose and ears may consequent in intracranial spread or orbital complications leading to high mortality or morbidity, yet early diagnosis and rapid management results in reduction of morbidity and mortality<sup>3</sup>.

Ear infections are one of the most frequent ENT disorders. A middle ear infection originates when bacteria enter through auditory tube and become trapped inside the inner ear. Children below the age of seven years are much more susceptible to otitis

media because the eustachian tube is shorter and at more of horizontal angle than in the adults and this is also because they have not yet developed the resistance to microbes as found in adults.<sup>4</sup> Acute otitis media occurs more commonly in children, and is most commonly caused by *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*<sup>5</sup>.

Nasopharyngeal colonization probably involves a complex combination of factors including host characteristics, host immune responses and direct competitive interactions between bacterial species<sup>6</sup>. *Staphylococcus aureus* is the main colonizer of nose<sup>7</sup>. Pharyngitis is the common of throat infections and is caused mainly by *Streptococcus pyogenes*<sup>8</sup>.

Rapid development of multidrug resistance by microorganisms to the available antimicrobial agents has increased the risk of infectious diseases to human health<sup>9</sup>. Biofilms formation and chronic antibiotic resistant ENT infections are also a threat in common. Detachment of cells, production of endotoxin, increased resistance to the host immune system and the development of organisms are biofilm processes that could initiate the infection process<sup>10</sup>. In order to avoid serious complications an active and prompt management of ENT infections is mandatory<sup>11</sup>. This study aimed to recognise the

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common ENT pathogens and the choices of antibiotics for their treatment.

## METHODOLOGY

A cross sectional observational study was done in the department of microbiology at The Children's Hospital and Institute of Child Health, Lahore. A total number of 217 samples were collected for the time period of 6 months from July 2012 to December 2012. ENT swabs were collected and cultured on Blood and MacConkey agar plates. Identification of bacteria was performed by Gram staining, biochemical tests (catalase, coagulase, DNase, oxidase, urease, citrate and triple sugar iron) and by using API system (API 20E and API 20NE).

Antimicrobial sensitivity testing was done on Mueller Hinton agar using Modified Kirby Bauer disc diffusion technique. Test inoculum was compared with 0.5 McFarland turbidity standard. The appropriate antimicrobial discs used for Gram negative bacteria were amoxicillin/clavulanic acid (AMC), amikacin (AK), cefuroxime (CXM), ceftazidime (CAZ), ceftriaxone (CRO), cefotaxime (CTX), ciprofloxacin (CIP), chloramphenicol (C), meropenem (MEM), piperacillin/tazobactam (TZP) and sulbactam/cefoperazone (SCF). Antimicrobial discs used for Gram positive bacteria were amoxicillin/clavulanic acid (AMC), amikacin (AK), ampicillin (AMP), penicillin (P), oxacillin (OX), ciprofloxacin (CIP), vancomycin (VA), piperacillin/tazobactam (TZP), ceftriaxone (CRO), cefotaxime (CTX), cefuroxime (CXM) and cefradine (CE). After putting appropriate antibiotics, the plates were incubated at 37°C for 16-18 hours. Interpretation of zone sizes of each antimicrobial disc against each organism was measured in mm as

sensitive, resistant or intermediate sensitive using interpretation chart of zone sizes.

## RESULT

Of the 217 samples, 136(62.67%) were found positive for pathogenic growth and 81(37.33%) samples were culture negative. Among 136 positive samples, single bacterial growth was present in 121 samples and 15 samples yielded double bacterial growth. The frequency of different organisms isolated was as follows: *Pseudomonas* 53(34.87%), *Staphylococcus aureus* 34(22.37%), *Proteus spp.* 18(11.84%) *Klebsiella spp.* 17 (11.18%), *E. coli* 9(5.92), *Enterobacter spp.* 6 (3.95%), *Acinetobacter spp.* 4(2.63%), *Citrobacter spp.* 3(1.97%), *Streptococcus pyogenes* 3(1.97%), *Streptococcus pneumoniae* 2(1.32%), *Haemophilus parainfluenzae* 2(1.32%) and *Stenotrophomonas maltophilia* 1(0.66%).

From the ear samples 119 (69.19%) cultures recovered the organisms. *Pseudomonas spp* (49), *Staphylococcus aureus* (34), *Proteus spp.* (18) and *Klebsiella spp.* (9) were the most frequent organisms. Other organisms associated with ear were *E. coli* (8), *Enterobacter spp.* (6), *Acinetobacter spp.* (3), *Citrobacter spp.* (3), *Haemophilus parainfluenzae* (2), *Streptococcus pneumoniae* (2) *Streptococcus pyogenes* (1) and *Stenotrophomonas maltophilia* (1). Of the 5 (45.45%) positive nasal samples, *Pseudomonas spp.* (2), *Staphylococcus aureus* (2) and *Klebsiella spp* (2) were isolated. In 12 (35.29%) positive throat samples, *Klebsiella spp.* (6), *Pseudomonas spp.* (2), *Streptococcus pyogenes* (2), *E. coli* (1) and *Acinetobacter spp.* (1) were isolated (Table 1).

Table 1: Frequency of bacterial pathogens isolates from ENT patients

Organisms	n	Ear swab (n=119)	Nasal swab (n=5)	Throat swab (n=12)
<i>Pseudomonas spp</i>	53(34.87%)	49	2	2
<i>Staphylococcus aureus</i>	34(22.37%)	32	2	0
<i>Proteus spp</i>	18(11.84%)	18	0	0
<i>Klebsiella spp</i>	17(11.18%)	9	2	6
<i>Escherichia coli</i>	9 (5.92%)	8	0	1
<i>Enterobacter spp</i>	6(3.95%)	6	0	0
<i>Acinetobacter spp</i>	4(2.63%)	3	0	1
<i>Citrobacter spp</i>	3(1.97%)	3	0	0
<i>Streptococcus pyogenes</i>	3(1.97%)	1	0	2
<i>Haemophilus parainfluenzae</i>	2(1.32%)	2	0	0
<i>Streptococcus pneumoniae</i>	2(1.32%)	2	0	0
<i>Stenotrophomonas maltophilia</i>	1(0.66%)	1	0	0

Table 2: Antibiotic susceptibility pattern of Gram positive organisms (n=39)

Antibiotics	Sensitive	Resistant
Pencillin	3 (7.69%)	36 (92.31%)
Ampicillin	3 (7.69%)	36 (92.31%)
Amikacin	37 (94.87%)	2 (5.13%)
Vancomycin	39 (100%)	0 (0%)
Oxacillin	26 (76.47%)	8 (23.53%)
Ciprofloxacin	35 (89.74%)	4 (10.26%)
Piperacillin/Tazobactam	26 (76.47%)	8 (23.53%)
Amoxicillin/Clavulanic acid	31 (79.49%)	8 (20.51%)
Ceftriaxone	31 (79.49%)	8 (20.51%)
Cefotaxime	26 (76.47%)	8 (23.53%)
Cefuroxime	31 (76.92%)	9 (23.08%)
Cefradine	26 (76.47%)	8 (23.53%)

Table 3: Antibiotic susceptibility pattern of Gram negative organisms (n=113)

Antibiotics	Sensitive	Intermediate Sensitive	Resistant
Meropenem	106(93.81%)	1(0.88%)	6(5.31%)
Tazobactam/Piperacillin	106(93.81%)	2(1.77%)	5(4.42%)
Sulbactam/Cefoperazone	109(96.46%)	2(1.77%)	2(1.77%)
Cefotaxime	36(31.86%)	3(2.65%)	74(65.49%)
Ceftazidime	85(75.22%)	1(0.88%)	27(23.90%)
Amoxicillin/Clavulanic acid	34(30.09%)	2(1.77%)	77(68.14%)
Ceftriaxone	44(38.94%)	4(3.54%)	65(57.52%)
Cefuroxime	26(23.01%)	1(0.88%)	86(76.11%)
Amikacin	96(84.96%)	2(1.77%)	15(13.27%)
Chloramphenicol	43(38.05%)	0(0%)	70(61.95%)
Ciprofloxacin	80(70.80%)	6(5.31%)	27(23.89%)

Antibiotic susceptibility pattern of Gram positive organisms showed 100% sensitivity to vancomycin followed by amikacin (94.87%). All the isolates of *Staphylococcus aureus* were resistant to penicillin and ampicillin but *Streptococcus spp* (*Streptococcus pyogenes* and *Streptococcus pneumoniae*) showed 60% susceptibility to these drugs. The isolates were also susceptible to ciprofloxacin (89.74%), amoxicillin\clavulanic acid (79.49%), cefuroxime (76.92%), ceftriaxone (79.49%), oxacillin (76.47%), piperacillin\tazobactam (76.47%), cefotaxime (76.47%) and cefradine (76.47%) (Table 2).

In Gram negative bacteria sulbactam/cefoperazone (96.46%), piperacillin/tazobactam (96.1%) and meropenem (93.81%) were the most effective drugs. The sensitivity of the isolates to other drugs included amikacin (84.96%), ceftazidime (75.22%) and ciprofloxacin (70.80%). The isolates were less susceptible to ceftriaxone (38.94%), chloramphenicol (38.05%), cefotaxime (31.86%), amoxicillin/clavulanic acid (30.09%) and cefuroxime (23.01%) (Table 3).

## DISCUSSION

In the present study ear, nose and throat swabs were analyzed to determine the frequency and antimicrobial sensitivity pattern of pathogenic bacteria of ear, nose and throat. According to the present

study a total number of 136(62.67%) pathogenic organisms were isolated from 217 samples. Single bacterial growth was present in 121(55.76%) samples while 15(6.91%) samples had double bacterial growth remaining 81(37.33%) samples had either no growth or normal flora microorganisms. These results are comparable with a study conducted in Benin city in which 272 samples were included, single bacterial growth was obtained in 165 (60.66%) samples and 69(25.36%) showed growth of two isolates<sup>12</sup>.

In our study *Pseudomonas spp.* 53(34.87%) were most frequently isolated organisms followed by the *Staphylococcus aureus* 34(22.37%), *Proteus spp.* 18(11.84%), *Klebsiella spp.* 17(11.18%) and *E. coli spp.* 9(5.92). A similar study performed in a Regional Hospital in Koso also revealed that the *Staphylococcus spp.*, *Pseudomonas* and *Streptococcus spp.* were the leading pathogens of ENT specimens<sup>13</sup>. Previous studies conducted in various regions also isolated the same microorganisms (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella spp.*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Proteus* and *E. coli*) with different frequencies<sup>14,15</sup>. The reason for such a wide number and frequency of pathogens might be poor hygienic conditions.

Gram positive organisms showed sensitivity to vancomycin (100%) amikacin (94.87%) and ciprofloxacin (89.74%) in the present study. An Indian study at Rajahmundry Andhra Pradesh reported sensitivity of *Staphylococcus aureus* to ciprofloxacin as 89% and gentamicin 76.5%.<sup>16</sup> In the present study Gram negative organisms were most sensitive to sulbactam/cefoperazone (96.46%), piperacillin/tazobactam (96.1%) and meropenem (93.81%). This is quite similar to a research work conducted in military hospital at Bahawalpur which reported 100% sensitivity against *Pseudomonas*.<sup>17</sup> A relatively less use of these antibiotics could be the reason for such a high sensitivity to these drugs.

*Streptococcus pyogenes* and *Streptococcus pneumoniae* were (60%) sensitive to penicillin and ampicillin but *Staphylococcus aureus* showed no sensitivity to these two drugs which is parallel to the research in China in which penicillin was sensitive to *Staphylococcus aureus* in 2.6% cases<sup>18</sup>. In the current study Gram negative organisms were found less sensitive to 2<sup>nd</sup> and 3<sup>rd</sup> generation cephalosporins (cefuroxime, ceftriaxone, cefotaxime), chloramphenicol and amoxicillin/clavulanic acid. A study conducted in Kashmir, India also reported less sensitivity (32%) of *Pseudomonas aeruginosa* to ceftriaxone<sup>19</sup>. Improper and overuse of antibiotics, low cost and easy accessibility and the development of enzymatic resistance may be the cause of reduce sensitivity and resistance<sup>20</sup>.

It is concluded with the present study that the *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus spp.* and *Klebsiella spp.* are the leading ENT pathogens in paediatric patients. Most of the isolates showed high resistance to cephalosporins. However they showed high effectiveness to meropenem, piperacillin/tazobactam and sulbactam/cefoperazone. The judicious use of antibiotics should be considered for the better treatment and to avoid the development of resistant organisms in ENT patients

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