

**Original Research Article:**

## **Aerobic bacteriological profile in patients of otitis media**

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**Abstract:**

Otitis media (OM) is an inflammatory disease of middle ear mucosa. Its significance lies in the dreaded complications and chronicity associated with the disease process. The present study was a cross sectional study conducted in the Department of Microbiology and ENT, AIMSRS, Bathinda, over a period of one year. 131 clinically diagnosed cases of otitis media fulfilling the clinical criteria, attending ENT department were studied. Two sterile swabs were used to collect ear discharge from each patient. First swab was used for direct microscopy by Gram staining and KOH mount. Second swab was used for culture onto MacConkey agar and Blood agar media. After incubation for 24 – 48 hours the plates were observed for growth, and isolates were confirmed based on standard microbiological procedure. Out of 131 patients, 110 (83.97%) were positive for culture and 21 (16.03%) were sterile. Total of 117 bacterial isolates were obtained from 110 culture positive samples. 71(60.68%) isolates were Gram negative and 46(39.32%) isolates were Gram positive. A total of six bacterial genera were isolated. Highest number of organism isolated was *Pseudomonas aeruginosa*, 45 (38.46%), followed by *Staphylococcus aureus* 39 (33.33%), *Escherichia coli* 14 (11.97%), *Klebsiella pneumoniae* 12 (10.26%), Coagulase negative *Staphylococcus* 4 (3.42%) and *Enterococcus faecalis* 3 (2.56%). CSOM was found in 84 (64.12%) patients over 47(35.88%) as ASOM. *Pseudomonas aeruginosa* 36(48%) was the predominant organism isolated in CSOM followed by *Staphylococcus aureus* 22(29.33%). Predominant organism isolated in ASOM was *Staphylococcus aureus* 17(40.48%) followed by, *Pseudomonas aeruginosa* 9(21.43%).

**Keywords:** Otitis media, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.

### **INTRODUCTION**

OTITIS MEDIA (OM) refers to a group of complex infections and inflammatory diseases affecting the middle ear. It has been broadly classified into two main types, Acute and Chronic.<sup>1</sup>

Acute suppurative otitis media (ASOM) is characterized by the rapid onset of signs of inflammation, specifically bulging and possible perforation of the tympanic membrane, fullness and erythema. Symptoms associated with inflammation such as otalgia, irritability and fever may also be present.<sup>1</sup>

Chronic suppurative otitis media (CSOM) is characterized by involvement of middle ear cleft in a long standing episode. It is found associated with ear discharge and a permanent perforation of tympanic membrane. A perforation becomes permanent due to covering of its edges by squamous epithelium and absence of spontaneous healing.<sup>2</sup>

Typical pathogens reach the middle ear through Insufflations of respiratory pathogens through the Eustachian tube from the Nasopharynx and Spread from the external ear canal inwards through a non-intact tympanic membrane.<sup>3</sup>

Bacteria, fungi and viruses are all potential pathogens in Otitis media. Knowledge of the true frequency of polymicrobial infection, particularly the extent of anaerobic involvement, is limited by differences in collection and culture techniques.<sup>4</sup>

#### **AIM AND OBJECTIVE**

**Aim:** To study the aerobic bacteriological profile in patients of otitis media in a tertiary care hospital.

**Objective:** To evaluate microbiological profile in cases of otitis media with ear discharge.

#### **MATERIAL AND METHODS**

Present study was a cross sectional study conducted in the Department of Microbiology in association with the Department of ENT, AIMS, Bathinda, over a period of one year (November 2016 – November 2017). All the patients with ear discharge in the Department of ENT were included in the study. Patient details and relevant information was recorded in a case record form. Patients fulfilling both clinical and microbiological criteria were considered into this study. The outcomes were studied by using frequencies and percentages.

Single use mini-tip sterile cotton swabs were used for sample collection and were transported in peptone water to maintain the swabs moist until being analyzed. All specimens were processed within 1 hour of collection. Two sterile swabs will be used to collect ear discharges from each patient under strict aseptic precautions. The first swab was processed for direct microscopy by Gram staining and KOH mount, for the presence of pus cells, epithelial cells, bacteria and yeast. The second swab was cultured aerobically on blood agar and MacConkey agar, and then incubated at 37°C for 24 to 48 hours.

Isolates were identified from colony characters on Blood agar medium, MacConkey agar medium and any special media used for growth. Organisms were further confirmed on the basis of various biochemical tests and other special tests required for that particular organism.

#### **RESULTS**

In the present study, 71 (54.20%) patients were in the age group of 21-40 years, followed by 33 (25.19%) in age group of 41-60 years, 16 (12.22%) in age group  $\leq 20$  years and 11 (8.39%) in the age group of  $\geq 61$  years. Maximum patients were from age group 21-40 years and minimum were in the age group  $\geq 61$  years.

In the present study, male predominance was seen i.e. 75 (57.25%) patients were male and 56 (42.75%) were female patients.

Out of 131(100%) patients of Otitis Media, 84 (64.12%) patients were of CSOM and 47(35.88%) were of ASOM.

In the present study, 110 (83.97%) specimens were positive for culture and 21 (16.03%) specimens were sterile for growth.

Out of 110 (83.97%) culture positive samples, 103 (78.63%) samples showed growth of single organism and 7 (5.34%) samples showed growth of mixed organisms (two organisms). Total 117 isolates were obtained from 110 culture positive samples.

On gram staining, out of 117 isolates, 71(60.68%) isolates were Gram negative organisms and 46(39.32%) isolates were Gram positive organisms.

In the present study, maximum number of organism isolated was *Pseudomonas aeruginosa*, 45 (38.46%), followed by *Staphylococcus aureus* 39 (33.33%), *Escherichia coli* 14 (11.97%), *Klebsiella pneumoniae* 12 (10.26%), Coagulase negative *Staphylococcus* 4 (3.42%) and *Enterococcus faecalis* 3 (2.56%).

Out of 110 (83.97%) pus samples, 7 (5.34%) pus samples showed mixed growth i.e. 4(57.14%) samples showed mixtures of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Mixtures of *Staphylococcus aureus* + *Pseudomonas aeruginosa*, *Staphylococcus aureus* + *Enterococcus faecalis* and *Escherichia coli* + Coagulase negative *Staphylococcus* from 1(14.29%) sample each.

Out of 75(64.12%) CSOM patients maximum number of organisms obtained were *Pseudomonas aeruginosa* 36(48%), followed by *Staphylococcus aureus* 22(29.33%), *Escherichia coli* 9 (12%), *Klebsiella pneumoniae* 5 (6.67%), *Enterococcus faecalis* 2 (2.67%) and Coagulase negative *Staphylococcus* 1 (1.33%).

Out of 42(35.88%) ASOM patients maximum number of organisms obtained were *Staphylococcus aureus* 17(40.48%) followed by, *Pseudomonas aeruginosa* 9(21.43%), *Klebsiella pneumoniae* 7 (16.67%), *Escherichia coli* 5 (11.90%), Coagulase negative *Staphylococcus* 3 (7.14%) and *Enterococcus faecalis* 1 (2.38%).

## DISCUSSION

The present study conducted on 131 Otitis Media patients, 110 (83.97%) were positive for culture and 21 (16.03%) were sterile. A total of six bacterial genera were isolated with *Pseudomonas aeruginosa*, 45 (38.46%) as the predominant organism followed by *Staphylococcus aureus* 39 (33.33%). So, an attempt is made to know the bacteriology of otitis media.

### Agewise distribution

In the present study, maximum numbers of patients were in the age group of 21-40 years, i.e. 71 (54.20%). The findings of present study are in correlation with Loy et al (2002)<sup>5</sup> as 41.1%, Vishwanath et al (2010)<sup>6</sup> as 37.2% and Das and Basumatari (2017),<sup>7</sup> as 42% patients were in the age group 21-40 years. Higher incidence of otitis media in adult age group may be due to regional variations and also because of multiple factors such as poor sanitation, unhygienic living, overcrowding, malnutrition, illiteracy and lack of health consciousness. Incidence of otitis media decreases as the age advances, this show no age is immune to this disease.

### Sexwise distribution

The present study shows male preponderance with otitis media in 75 (57.25%) over 56 (42.75%) females. These findings are correlated with the studies done by Dhingra et al (2016)<sup>8</sup> as 53% and Aliyu et al (2017)<sup>9</sup> as 59.20% male patients. The male predominance may have been because of lifestyles that exposed them to more risks compared to females such as being in contact with contaminated water in pools or rivers and probably because males may have more health seeking behaviors when ill than females.

### Distribution of type of otitis media

In the present study, majority of patients showed chronic (CSOM) type of otitis media, in 84 (64.12%), whereas acute (ASOM) type of Otitis media was seen in 47 (35.88%) only. This finding is in accordance with Haneefa et al (2015)<sup>10</sup> as CSOM in 66.54% patients and Ujwala et al (2016)<sup>11</sup> as 66.86 % patients. It is predicted that, if otitis media with effusion persist for weeks to months after onset of acute otitis media, recurrent episodes of ASOM

occurred, leading in chronic cases. This may be the reason for increasing incidence of CSOM, as also found in present study.

#### **Culture results in accordance with type of growth**

In the present study, analysis of the total of 131 specimens revealed that 110 (83.97%) were culture positive and 21 (16.03%) were culture sterile. Out of 110 positive cultures, 103 (78.63%) were with pure growth and mixed growth was seen in 7 (5.34%). Overall 117 isolates were obtained from 131 samples.

The culture results correlates with Wadile et al (2015),<sup>12</sup> found 86.81% with pure growth, 13.18% with mixed growth and 10% with no growth.

Sterile cultures can be attributed to anaerobic bacteria, non-bacterial organisms, presence of antimicrobial enzymes i.e. lysozyme alone or in combination with immunoglobulins that suppress the bacterial growth. Polymicrobial growth in ear samples could be because of contamination from the external auditory meatus.

#### **Culture results based on gram staining**

In the present study, Gram negative organisms accounted for 71(60.68%) and Gram positive organisms were 46(39.32%). It is mostly seen that Gram negative organisms outnumber Gram positive organisms in otitis media as reported by Shetty et al. (2014)<sup>13</sup> as 51.22 %, and Ghosh et al (2015)<sup>14</sup> as 54.61%, which is comparable to present findings.

Gram negative organisms inhabit human gut and body as saprophyte, are found to be transferred to ear mostly through environmental and personal unhygienic practices. The higher incidence of Gram negative organisms in our study could be because of fecal contamination of water, which occurs during bathing or swimming.

#### **Bacteriological profile**

In the present study, 6 bacterial genera were isolated in 110 positive cultures. The most common organism isolated was *Pseudomonas aeruginosa* 45 (38.46%) followed by *Staphylococcus aureus* 39 (33.33%), *Escherichia coli* 14 (11.97%), *Klebsiella pneumoniae* 12 (10.26%), Coagulase negative *Staphylococcus* 4 (3.42%) and *Enterococcus faecalis* 3 (2.56%).

*Pseudomonas aeruginosa* was the predominant organism isolated in studies reported by, Shetty et al (37.80%)<sup>13</sup> and Ghosh et al (40.76%)<sup>14</sup>, which correlates at par with our series.

The occurrence of *Pseudomonas aeruginosa* as the predominant offending organism could be attributed to several factors. *Pseudomonas aeruginosa* survives competition with other pathogens could be due to minimum nutritional requirements, its relative resistance to antibiotics and its armamentarium of antibacterial products i.e., pyocyanin and bacteriocin. Apart from the above said reasons, it uses the pili to attach to the necrotic or diseased epithelium of middle ear. Once attached, the organism produces enzymes like proteases, lipopolysaccharides, etc. to elude from normal defense mechanism of the body required for fighting infections. In addition, the organism acts as an opportunistic pathogen, flourishes in external auditory canal and causes suppurative disease.

The second most common organism isolated was *Staphylococcus aureus* (33.33%) and find correlation with studies conducted by, Shetty et al. (30.49%)<sup>13</sup> and Ghosh et al. (28.46%)<sup>14</sup>.

The frequency of *Staphylococcus aureus* in the middle ear infections can be attributed to their ubiquitous nature and high carriage of resistant strains in external auditory canal and upper respiratory tract.

In the present study various other bacteria isolated were, *Escherichia coli* 14 (11.97%), *Klebsiella pneumoniae* 12 (10.26%), Coagulase negative *Staphylococcus* 4 (3.42%) and *Enterococcus faecalis* 3 (2.56%). The similar organisms have been found to be associated with OM in studies conducted by Shetty et al. (2014)<sup>13</sup> and Ghosh et al. (2015).<sup>14</sup>

Organisms like *Escherichia coli* and *Klebsiella pneumoniae* become opportunistic pathogens in the middle ear when resistance is low. Although CONS are generally considered as non-pathogenic, their association in some cases can be attributed to the extreme lowering of resistance in middle ear due to invasion by other organisms. Under these circumstances they assume pathogenic role either singly or more often in combination with other organisms.

#### **Distribution and percentage of mixed isolates**

In present study, out of 110 samples, 7 (5.34%) showed mixed growth. 4(57.14%) samples showed mixtures of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. 1(14.29%) each showed mixtures of *Staphylococcus aureus* + *Pseudomonas aeruginosa*, *Staphylococcus aureus* + *Enterococcus faecalis* and *Escherichia coli* + Coagulase negative *Staphylococcus*.

Aliyu et al (2017)<sup>9</sup> identified mixed bacterial infections in 6 (8.5%) of the samples. *Staphylococcus aureus* and *Pseudomonas aeruginosa* mixed culture were frequent than others.

Mixed growth of organisms are usually associated with contamination from external meatus or unhygienic practices.

#### **Association between isolates and type of otitis media**

##### **CSOM and various isolates**

In present study, out of 75(64.12%) CSOM patients, maximum number of organisms obtained were *Pseudomonas aeruginosa* 36(48%), followed by *Staphylococcus aureus* 22(29.33%), *Escherichia coli* 9 (12%), *Klebsiella pneumoniae* 5 (6.67%), *Enterococcus faecalis* 2 (2.67%) and Coagulase negative *Staphylococcus* 1 (1.33%).

Haneefa et al (2015)<sup>10</sup> observed that among the bacterial isolates in CSOM, *Pseudomonas aeruginosa* was the predominant organism that accounted for 62 (43.36%). *Staphylococcus aureus* was the only Gram positive bacterial pathogen isolated 51 (33.65%).

##### **ASOM and various isolates**

In present study, out of 42(35.88%) ASOM patients maximum number of organisms obtained were *Staphylococcus aureus* 17(40.48%) followed by, *Pseudomonas aeruginosa* 9(21.43%), *Klebsiella pneumoniae* 7 (16.67%), *Escherichia coli* 5 (11.90%), Coagulase negative *Staphylococcus* 3 (7.14%) and *Enterococcus faecalis* 1 (2.38%).

These findings are in accordance with Haneefa et al (2015)<sup>10</sup> who observed that among the bacterial isolates in ASOM, *Staphylococcus aureus* was the predominant organism that accounted for 44(50.58%), followed by *Pseudomonas aeruginosa*, 28 (32.18%).

The abundance of *Staphylococcus aureus* in ASOM might be as a result of close proximity of the middle ear and external ear, where they live as normal flora. They ascend from the nasopharynx to the middle ear via eustachian tube, during acute otitis media, where they become pathogens.

It is further observed that if ASOM persist for weeks to months after onset, recurrent episodes of ASOM occurred, landing in CSOM. *Pseudomonas aeruginosa* reign as prime offender in CSOM because it can thrive well in the ear

environment and is difficult to eradicate. *Pseudomonas aeruginosa* evades host defense mechanisms easily and damages the tissues.

## CONCLUSION

The present study conducted on 131 Otitis Media patients, 110 (83.97%) were positive for culture and 21 (16.03%) were sterile. Out of 110 positive cultures, 103 (78.63%) were with pure growth and mixed growth was seen in 7 (5.34%). Overall 117 isolates were obtained from 131 samples. A total of six bacterial genera were isolated with *Pseudomonas aeruginosa*, 45 (38.46%) as the predominant organism followed by *Staphylococcus aureus* 39 (33.33%). Maximum patients were from age group 21-40 years, 71 (54.20%) with male: female ratio as 1.34:1. *Pseudomonas aeruginosa* 36(48%) was the predominant organism isolated in CSOM followed by *Staphylococcus aureus* 22(29.33%). Predominant organism isolated in ASOM was *Staphylococcus aureus* 17(40.48%) followed by, *Pseudomonas aeruginosa* 9(21.43%).

Since Otitis Media can cause significant morbidity, knowledge of the pathogens for Otitis Media helps in the appropriate and timely treatment which can minimize the complications.

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