

## MICROORGANISMS PROFILE AND ANTI-MICROBIAL SENSITIVITY IN CHRONIC SUPPURATIVE OTITIS MEDIA

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Date Submitted: January 25, 2019

Date Revised: October 25, 2019

Date Accepted: October 27, 2019

### ABSTRACT

**OBJECTIVE:** To determine the frequency of common microorganisms involved in chronic suppurative otitis media (CSOM) and their antibiotic sensitivity.

**METHODS:** This cross sectional study of 776 patients suffering with CSOM was conducted at Otolaryngology Department, Ayub Teaching Hospital, Abbottabad from 5<sup>th</sup> May 2017 to 5<sup>th</sup> November 2018. Patients fulfilling the inclusion criteria were enrolled for otoscopic examination. Sterile cotton swabs were used to collect pus samples and were sent to microbiological analysis and antibiotics sensitivity.

**RESULTS:** The age ranges of the patients were from 13-73 years, with a mean age of 28.98±13.40 years. The incidence of CSOM was highest in 21-30 years age group (294~37.9%). In a total 776 CSOM patients, only 501 (64.56%) of the samples showed microbial growth. In aerobic isolates, the most common bacterium was *Pseudomonas aeruginosa* (30.7%) followed by *Staphylococcus aureus* (11.1%) including 6.7% MRSA. Anaerobes were isolated in 1.2% of the samples in which bacteriodes were being the most common. Samples (1.8%) showed fungal growth and yielded only *Candida spp* while 0.5% samples were positive for acid fact bacilli. In antimicrobial sensitivity investigation *Pseudomonas aeruginosa* showed highest sensitivity to piperacillin/tazobactam (84%), to levofloxacin (25.21%), to ciprofloxacin (20.59%). The highest sensitivity of *Staphylococcus aureus* was investigated for piperacillin/tazobactam and ceftazidime with 91.18%, and showed no sensitivity to ciprofloxacin and levofloxacin.

**CONCLUSION:** *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most common bacteria. Both show increasingly high resistance to quinolones and amino glycosides, but both isolates are sensitive to piperacillin/tazobactam, imipenam, cefoperazone/salbactam and ceftazidime.

**KEY WORDS:** Microorganisms (MeSH); Sensitivity (MeSH); Otitis Media, Suppurative (MeSH); Otitis Media (MeSH); Chronic suppurative otitis media (Non-MeSH); Antibiotics (MeSH); *Pseudomonas* (MeSH); *Escherichia coli* (MeSH); *Staphylococcus* (MeSH).

**THIS ARTICLE MAY BE CITED AS:** Ibrahim M, Ali SM, Shah MI, Khan MA. Microorganisms profile and anti-microbial sensitivity in chronic suppurative otitis media. Khyber Med Univ J 2019;11(4):248-52. DOI: 10.35845/kmu.2019.18958

### INTRODUCTION

Chronic suppurative otitis media (CSOM) is one of the commonest infectious disease of ear both in the developing and developed countries.<sup>1-3</sup> The disease incidence is higher in the

developing countries especially in those countries having poor socio-economic status because of unsatisfactory hygiene, nutritional deficiency, frequent upper respiratory tract infections, paucity of health education, under resourced health care, overcrowding, facial anomalies and

immunosuppression.<sup>2-9</sup> CSOM is a worldwide problem and individuals with all range of age are affected but particularly it is dominant in childhood.<sup>1-5, 10,11</sup> It affects approximately 2% of the overall population with urban: rural ratio of 1:2.<sup>1,8,10,12</sup> Chronic suppurative otitis media is actually chronic inflammation and/or infection of the middle ear cleft (tympanic cavity, eustachian tube, aditus, antrum and mastoid air cells) having continuous or recurrent discharge of 3 months or more through a perforated tympanic membrane (TM).<sup>1-4, 6-8, 13-16</sup> It is a disease of multiple etiology.<sup>17-19</sup> The most common pathogenic factors in the disease are the bacterial infection and dysfunction of the eustachian tubes.<sup>16,20</sup> The common symptoms of the disease are itching, ear discharge, deafness, pain and rarely fever.<sup>2</sup> Depending upon the involvement of pars tensa or flaccida of the TM by the disease process, CSOM is divided into tubotympanic and attic-antral types respectively.<sup>6,9</sup> Cholesteatoma may or may not be associated with CSOM.<sup>1,4</sup> It causes a diversity of intracranial and extracranial complications because it is potentially a serious disease.<sup>2,15</sup> The infection can extend beyond the confines of middle ear and can cause complications like deafness, paralysis of facial nerve, mastoid abscess, thrombophlebitis of the lateral sinus, labyrinthitis, petrositis, intracranial abscess and meningitis.<sup>3,6,12,15</sup> Hearing loss occurs in 50% cases of CSOM which is the most common complication.<sup>2, 6,12,17</sup> This may results into communication problems, hindering professional life and social interaction.<sup>12,21,22</sup> The most common micro-organisms previously found in chronic suppurative otitis media include, *Pseudomonas aeruginosa* (45.9%), *Staphylococcus aureus* (26.4%), *Proteus mirabilis* (8%), *Klebsiella pneumoniae* (2%), *Escherichia coli* (6.8%), *Aspergillus sp* and *Candida sp* (3.7%).<sup>23</sup>

However the diversity and proportion of microorganisms existing in CSOM depend upon the geographical locations. Different geographical areas are having different profile of the microorganisms associated CSOM.<sup>5,23,24</sup> Previous studies have reported different proportion of microorganisms

**TABLE I: LIST OF MICROORGANISMS TO BE CULTURED**<sup>26-28</sup>

Microorganisms	Identification
<i>Pseudomonas aeruginosa</i>	Gram -ive rods, lactose non-fermenter, oxidase +ive, producing blue green colonies on blood and chocolate agar.
<i>Staphylococcus aureus</i>	Gram +ive, clusters of cocci, catalase +ive, coagulase +ive, producing golden yellow colonies on blood agar.
<i>Proteus mirabilis</i>	Gram -ive rods, lactose non-fermenter, oxidase -ive, producing swarming growth on blood agar.
<i>Escherichia coli</i>	Gram -ive rods, lactose fermenter (fast), producing pink purple dry colonies on MacConkey's agar
<i>Klebsiella pneumoniae</i>	Gram -ive rods, lactose fermenter (fast), producing mucoid colonies on MacConkey's agar.
Fungi	Candida sp - white color 2-3mm colonies on Sabouraud's agar. On microscopy, these are oval shaped & pseudohyphae are observed in germ tube test at 37°C. Aspergillus sp-molds, not dimorphic producing green or black colonies on Sabouraud's agar depending upon species after 3 days at room temperature.

**TABLE II: LISTS OF ANTIBIOTICS TO BE CHECKED SENSITIVITY AGAINST EACH CULTURE 26-28**

Antibiotics	Zone of inhibition—interpretive criteria nearest whole mm			
	Disk contents	Sensitive	Intermediate	Resistant
Tazobactam/piperacillin	100/10µg	≥ 21	15-20	≤ 14
Amoxicillin/clavulanic acid	20/10µg	≥ 18	14-17	≤ 13
Ceftazidime	30µg	≥ 18	15-17	≤ 14
Ceftriaxone	30µg	≥ 21	14-20	≤ 13
Cefixime	5µg	≥ 19	16-18	≤ 15
Ciprofloxacin	5µg	≥ 21	16-20	≤ 15
Levofloxacin	5µg	≥ 17	14-16	≤ 13
Gentamicin	10µg	≥ 15	13-14	≤ 12
Amikacin	30µg	≥ 17	15-16	≤ 14
Vancomycin	30µg	≥ 16	14-16	≤ 13
Imipenam	10µg	≥ 19	16-18	≤ 15
Cefoperazone/salbactam	75µg	≥ 21	16-20	≤ 15
Chloramphenicol	30µg	≥ 18	13-17	≤ 12
Clindamycin	2µg	≥ 21	15-20	≤ 14
Tobramycin	10µg	≥ 15	13-14	≤ 12

like Shresta, et al.<sup>5</sup> showed that the most common bacterial isolate is *Staphylococcus aureus* (32.2%) followed by *Pseudomonas aeruginosa* (26.9%) which is a contrast to so many other studies. This contradiction in studies might be due to difference of organisms in various geographical areas and the effect of climate.<sup>5</sup>

As the sensitivity pattern of the causative organisms CSOM is changing rapidly in our region where over the counter use of antibiotics is very common, we planned this study to find out the most common microorganisms involved and their antibiotic sensitivity in patients with CSOM in our region.

## METHODS

This cross sectional study comprises of 776 CSOM patients with consecutive non-probability sampling was conducted at outdoor patient department of ENT, head and neck surgery of Ayub Teaching Hospital, Abbottabad from 5<sup>th</sup> May 2017 to 5<sup>th</sup> November 2018. Both, male and female patients with age of above 12 years, having active ear discharge, ear discharge of more than 3 months, unilateral or bilateral ear discharge, were included in this study, while patients having ear discharge with aural polyp, external auditory canal pathology or any existing ear malignancy, having had mastoid surgery in the

preceding 12 months and those who had used antibiotics in the last 7-10 days were excluded from study. Informed consent was taken from all patients. The biodata of the patients along with hospital registration number were entered on proforma. The patients were assessed initially by history and otoscopic examination. Pus samples collected on sterile swabs for culturing of microorganisms from the discharging ear(s) were sent to consultant microbiologist in the hospital's pathology laboratory for processing. Lab reports of the pus swab collected from the discharging ear showing the bacterial spectrum and their sensitivity to antibiotics were reviewed and data entered in the proforma.

For aerobes, samples were inoculated onto chocolate agar and blood agar whereas medium of Robertson's cooked meat and neomycin blood agar were used for anaerobes. Sabouraud's dextrose agar was used for fungal culture. The bacterial isolates were identified using gram staining, colony morphology, coagulase, catalase & oxidase tests while fungal species were identified by India ink preparation & microscopy, and colony morphology.<sup>25-27</sup> The details of each aerobic bacteria and fungi is given in the Table I. In case of anaerobic bacterial culture processing, before inoculation Robertson's cooked meat medium (pieces of fat free minced cooked meat of ox heart) is boiled to make it oxygen free (by reducing agents present in meat like unsaturated fatty acids, glutathione & cysteine). Neomycin is added to medium to selectively inhibit the growth of gram-negative microorganisms. After inoculation the medium is covered with a layer of sterile liquid paraffin oil to prevent entry of oxygen to medium.

Mueller Hinton agar was used for testing of antibiotic sensitivity by modified method of Kirby-Bauer disc diffusion, in which different types of antibiotics saturated on small disks of filter paper, were placed onto the bacterial lawn. After incubation period of 18-24 hours, the plate was examined to see the bacterial growth whether it was inhibited (or not) by measuring clear zone of no bacterial growth around the antibiotic disk.<sup>25-27</sup>

All the data was entered in SPSS version 19.0 and analyzed. To calculate frequencies, percentages, means and standard deviation for both qualitative and quantitative variables, descriptive statistics were applied. The qualitative variables or categorical data like culture & sensitivity findings were expressed as frequency and

**TABLE III: PERCENTAGE OF DIFFERENT MICROORGANISMS ISOLATED FROM CSOM (n=776)**

Microorganisms isolated		Percentage
Positive growth (n=501)	Monomicrobial growth	58.11
	Polymicrobial growth	6.4
	Aerobes	54.8
	<i>Pseudomonas aeruginosa</i>	30.7
	<i>Staphylococcus aureus</i>	11.1
	<i>Escherichia coli</i>	5.7
	<i>Proteus spp</i>	3.9
	<i>Klebsiella pneumoniae</i>	3.4
	Anaerobes	1.2
	Bacteriodes	0.8
	<i>Peptostreptococcus</i>	0.3
	<i>Prevotellamelaninogenica</i>	0.1
	Fungi ( <i>Candida spp</i> )	1.8
	Acid fast bacilli (AFB)	0.5
No growth (n=275)		35.44

**TABLE IV: PERCENTAGE OF SENSITIVITY OF DIFFERENT BACTERIA ISOLATED FROM CSOM TO DIFFERENT ANTIBIOTICS (n=501)**

Antibiotic	Bacteria Isolated (Percentage)					
	<i>P. aeruginosa</i>	<i>S. aureus</i>	MRSA	<i>E coli</i>	<i>Proteus spp</i>	<i>K. Pneumoniae</i>
Piperacillin/Tazobactam	84	91.18	--	100	100	100
Imipenam	65.13	--	--	97.73	--	80.77
Cefoperazone/Salbactam	61.34	67.65	--	--	93.33	--
Amikacin	59.24	--	--	18.18	--	69.23
Ceftazidime	58.4	91.18	0	38.64	86.67	38.46
Tobramycin	44.12	--	--	--	63.33	53.85
Levofloxacin	25.21	0	0	47.73	100	--
Ciprofloxacin	20.59	0	28.85	--	36.67	--
Ceftriaxone	11.76	55.88	0	--	70	34.62
Amoxicillin/Clavulanic acid	--	64.71	--	--	--	--
Vancomycin	--	--	84.62	--	--	--
Chloramphenicol	--	--	73.08	--	--	--

percentages while quantitative variables or numerical data such as age were expressed as mean ± standard deviation.

**RESULTS**

After processing of total 776 ear swabs, only 501 (64.56%) samples showed microbial growth while 275 (35.4%) samples yielded no growth. Monomicrobial growth was seen in 451 (58.11%) samples whereas 50 (6.4%) samples showed poly-microbial growth. Anaerobes were isolated in 6 (1.2%) of the samples. Only 9 (1.8%) samples showed fungal growth and 2 (0.5%) samples were positive for AFB.

The age ranges of the patients were from 13 to 73 years, with a mean age of 28.98 ± 13.40 years. The incidence of CSOM was highest in 21-30 years age group (294 ~ 37.9%) followed by age group of 12-20 years 247 (31.8%). Least common age group was 50+ years 59 (7.6%). In this study, the percentage of male patients was 426 (54.9%) while female percentage was 350 (45.1%) with a male to female ratio of 1.21:1. The duration of ear discharge was 3-12 months in 345 (44.5%) patients, 13-24 months in 210 (27.1%) patients, 25-36 months in 82 (10.6%) patients and 36+ months in 139 (17.9%) patients. Most of the patients were having continuous ear discharge 451

(58.1%) followed by intermittent ear discharge 203 (26.2%) and then recurrent ear discharge 122 (15.7%). The most common TM perforation was central in 653 (84.1%) ears followed by subtotal 50 (6.4%), then total 37 (4.8%), attic 21 (2.7%) and the least common perforation was marginal in 15 (1.9%) ears.

The details of positive sample as per organism based are shown in Table III. The sensitivity of different drugs with respect organisms is highlighted in detail as per Table IV.

**DISCUSSION**

CSOM is a genuine healthcare issue around the world, not only because of the disease for patient and their family but it also puts significant financial burden on healthcare system. In this study 21-30 years of age group was having the highest incidence of CSOM (37.9 %) followed by age group of 12-20 years (31.8%) which is consistent with the results of Kumar H, et al.,<sup>8</sup> Iqbal K, et al.<sup>23</sup> and Mansoor T, et al.<sup>24</sup> in contrast to Prakash R, et al.,<sup>6</sup> Jayanthi SR, et al.<sup>7</sup> and Arvind N, et al.<sup>15</sup> who reported that CSOM is more common in the age group of below 20 years. CSOM is more common in male with a male to female ratio of 1.21:1 which is consistent with most of the studies<sup>1,3,8,14,16,23</sup> but Meyer E, et al., Prakash R, et al., Jayanthi SR, et al. and Mansoor T, et al. reported that CSOM is more common in female as compared to male.<sup>6,7,13,24</sup> The males having more exposed pattern of life may have been results in their predominance.<sup>8</sup> Most of the studies showed monomicrobial isolates to be more common as compared to polymicrobial isolates<sup>6,7,16,24</sup> which is consistent with our study. Central perforation of TM was more common (84.1%) and most of the studies also showed that central perforation was more common<sup>5,23</sup> but Nikakhlagh S, et al.<sup>14</sup> said that marginal perforation of TM associated with CSOM was more common which is in contradiction to our study.

This study showed that *Pseudomonas aeruginosa* and *S. aureus* were the major isolates involved in CSOM. The sensitivity pattern of these organisms showed that they were more sensitive to piperacillin/tazobactam, imipenam, cefoperazone/salbactam but increasingly becoming more resistant to quinolones and aminoglycosides.

Among aerobic isolates, the most common was *Pseudomonas aeruginosa* (30.7%) followed by *S. aureus* (11.1%) (including

6.7% MRSA) which is consistent with most of the national and international studies<sup>2-5,15,23,24</sup> but studies from India, Iran and South Africa showed that *S. aureus* was the most frequent bacterium in CSOM as compare to *Pseudomonas aeruginosa*.<sup>6,13,14,16,28</sup> While other 2 researches carried out in Nigeria and India showed that the commonest isolate was *Pseudomonas* followed by *Klebsiella*.<sup>1,7</sup>

In this study *Pseudomonas aeruginosa* showed highest sensitivity to piperacillin/tazobactam (84%) followed by imipenam (65.13%) then cefoperazone/salbactam (61.34%), amikacin (59.24%), ceftazidime (58.40%), tobramycin (44.12%). The sensitivity to levofloxacin was 25.21%, ciprofloxacin 20.59% and ceftriaxone 11.76%. In recent past *Pseudomonas aeruginosa* has showed decreasing pattern in sensitivity to quinolones worldwide.<sup>29</sup>

In Turkey a study was conducted in 1996 in which only 6% *Pseudomonas aeruginosa* isolates were resistance to ciprofloxacin<sup>30</sup> while in another study conducted in South Korea in 2004, 100% of the isolates showed resistance to ciprofloxacin.<sup>31</sup> The studies conducted in different areas of Pakistan showed more than 80% sensitivity.<sup>4,23,24</sup> In contrast, our study showed less than 26% sensitivity to quinolones. The deteriorating sensitivity pattern may be due to a variety of factors like imprudent antibiotic use, inadequate dosage, developing enzymatic resistance of organism towards quinolones and easy availability,<sup>4</sup> same increasing resistances of aminoglycosides have also been noted against *Pseudomonas aeruginosa*.

The highest sensitivity of *S. aureus* (other than MRSA) was to piperacillin/tazobactam and ceftazidime (91.18%) followed by cefoperazone/salbactam (67.65%), amoxicillin/clavulanate (64.71%), ceftriaxone (55.88%) and 0% sensitive to ciprofloxacin and levofloxacin. The sensitivity pattern of amoxicillin/clavulanate against *S. aureus* in our study is in accordance with other studies,<sup>2,16,28</sup> but the sensitivity pattern of *S. aureus* shows a downward trend to quinolones for the last few years. A study conducted in Iran in 2011 showed 85.4% sensitivity of *S. aureus* to ciprofloxacin,<sup>28</sup> whereas in Karachi, Pakistan a study conducted in 2013 showed 60% sensitivity to ciprofloxacin<sup>2</sup> and other study carried out in India in 2014 showed 55.3% sensitivity to ciprofloxacin<sup>16</sup> while in our study *S. aureus* showed 0% sensitivity to

ciprofloxacin.

## CONCLUSION

The most microorganisms *Pseudomonas aeruginosa* and *Staphylococcus aureus* are increasingly becoming more resistant to quinolones and aminoglycosides, but in our study both isolates were more sensitive to piperacillin/tazobactam, imipenam, cefoperazone/salbactam and ceftazidime.

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## AUTHORS' CONTRIBUTIONS

Following authors have made substantial contributions to the manuscript as under:

**MI:** Conception and study design, drafting the manuscript, critical review, final approval of the version to be published

**SMA, MIS & MAK:** Acquisition, analysis and interpretation of data, drafting the manuscript, final approval of the version to be published

*Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.*

## CONFLICT OF INTEREST

Authors declared no conflict of interest

## GRANT SUPPORT AND FINANCIAL DISCLOSURE

NIL



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