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Original Research Article

Aerobic bacteriology of chronic suppurative otitis media and its antibiotic susceptibility pattern in a tertiary care hospital, Bagalkot

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Abstract

Background: Chronic Suppurative Otitis Media (CSOM) remains one of the most common childhood chronic infectious diseases worldwide, affecting diverse racial and cultural groups both in developing and industrialized countries. India is one of the countries with highest CSOM prevalence (>6%) where urgent attention is needed. Infection usually results from bacterial, fungal and in some cases secondary to other viral infections like upper respiratory tract infections and tuberculosis. It is a common cause of conductive deafness which may lead to delayed development of speech and language in children and fatal intracranial complications. Change in the bacteriological scenario with indiscriminate use of antimicrobial agents has been associated with the emergence of multiple drug resistant strains. Therefore, the present study was carried out to determine the aerobic bacteria involved and their antibiotic susceptibility pattern in patients with CSOM. **Methods:** Ninety-three clinically diagnosed cases of CSOM of all age groups and both the sexes attending ENT OPD and admitted in ENT wards were studied. Two swabs were taken from each patient, one for Gram's staining and the other one for culture onto blood agar and MacConkey agar. After isolation, Gram stain, and biochemical reactions were done according to the procedures. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method. **Results:** 90.3% of the total samples yielded bacterial growth, while 9.68% were sterile. A total number of 10 different bacterial species were isolated from 89 isolates. The most common organism isolated was *P.aeruginosa* (30.3%) followed by *S. aureus* (27%), *P. mirablilis* (11.2%), *K. pneumoniae* (9%), *E. coli* (5.6%), *CONS* (5.6%), *C. freundi* (5.6%), Acinetobacter *spp* (2.2%), *S. pyogenes* (2.2%) *and P.vulgaris* (1.1%). Among Gram negative isolates the most sensitive drugs were amikacin and ofloxacin with 93.1% sensitivity, followed by piperacillin + tazobactam (88.9%) and ciprofloxacin (81%). Amoxyclav was the most resistant antibiotic (10.3%). The most effective antibiotic against gram positive bacteria in the present study was found to be ofloxacin and vancomycin (both 100% sensitivity), followed by chloramphenicol (96%). Ampicillin was the most resistant antibiotic, only 19.4% organisms were sensitive to it. **Conclusion:** The most common organism isolated was *P.aeruginosa* followed by *S. aureus.* The most effective antibiotic against gram positive bacteria was found to be ofloxacin. Among gram negative isolates the most sensitive drugs were amikacin and ofloxacin.

Keywords: CSOM, *Pseudomonas aeruginosa*, Ofloxacin.

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Introduction

Chronic suppurative otitis media (CSOM) has been an important cause of middle ear disease since prehistoric times [1]. CSOM has been a source of a tremendous health predicament since time immemorial and even today it is immensely intricate both for patients and an otologist to deal with [2]. CSOM is defined as the persistent inflammation of the middle ear or mastoid cavity, characterized by recurrent or persistent ear discharge (otorrhoea) over 2-6 weeks through a perforation of the tympanic membrane [3]. India is one of the countries with highest CSOM prevalence (>6%) where urgent attention is needed [4]. The disease is mainly classified into two types: tubotympanic and atticoantral depending on whether the disease process affects the pars tensa or the pars flaccida of the tympanic membrane [5]. The most common organisms isolated are *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella spp, Proteus mirabilis and Escherichia coli* among aerobe [6]. CSOM has received considerable attention, not only because of its high incidence and chronicity, but also because of issues such as bacterial resistance and ototoxicity with both topical and systemic

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antibiotics [7]. The widespread use of antibiotics has precipitated the emergence of multiple resistant strains of bacteria which can produce both primary and post-operative complications [8]. Though the treatment of CSOM is controversial and subject to change, particularly in developing countries, the antibiogram of these organisms have been reported to vary with time and geographical area, probably due to the use and abuse of antibiotics. Hence the need for periodic update of antibiogram for effective chemotherapy and management of CSOM cannot be over emphasized [9].

Therefore, the present study was undertaken to know the new trend of prevalence of CSOM, different bacteria causing CSOM and antibiogram of bacterial agents in CSOM among the people in the northern part of Karnataka.

Objectives

1. To isolate and to identify aerobic bacteria from CSOM cases.
2. To assess the antibiotic susceptibility pattern of the isolates.

Methods

Study settings

1. Department of Microbiology, S. Nijalingappa Medical College and Hanagal Shri kumareshwar Hospital and Research Centre, Bagalkot.
2. Department of ENT, S. Nijalingappa Medical College and Hanagal Shri kumareshwar Hospital and Research Centre, Bagalkot.

Study design: Prospective study
Duration of study: 1 year

Sampling method: Census method of sampling
Sample size: A total of 93 patients with CSOM of all age groups and both sexes attending ENT OPD and those admitted in ENT IPD were selected for the study.

Inclusion criteria

1. Patients diagnosed as suffering from CSOM after thorough clinical evaluation by an ENT surgeon.
2. Patients who are not on antibiotic (both topical and systemic) treatment for a minimum of 24 hours prior to sample collection.

Exclusion criteria

1. Patients suffering from CSOM who are on systemic antibiotics.
2. Patients who are on topical medications to the ear.

Statistical analysis: Data obtained were consolidated, statistically evaluated using SPSS software version 16 and results obtained were represented using charts and tables.

Methodology: The study was conducted after approval by the human ethics committee, S. Nijalingappa Medical College and Hanagal Shri kumareshwar Hospital and Research Centre, Bagalkot.

Collection of sample: Ear discharge was collected under aseptic precautions from the affected ear of CSOM cases. Excess discharge was mopped up from external auditory canal and it was cleaned with 70% alcoholic first and was allowed to act for 30-40 seconds to achieve sterile area. Then with two sterile swabs specimen was collected. One was for Gram's stain and the other one was for culture. Both swabs were processed immediately in the laboratory.

Direct microscopy: With one swab, a smear was made on a clean grease free glass slide and was fixed by heating. Gram's staining was done for the smears so made and observed under oil immersion microscope. The stained smear was screened carefully for the presence of bacteria and their Gram reaction, shape, size, and arrangement and also for the presence or absence of pus cells.

For aerobic culture

A. Inoculation on culture media: The second swab was inoculated onto dried plates of 5% sheep blood agar and MacConkey agar by rolling the swabs over the agar to make a primary well and then streaking from the primary inoculation using a sterile bacteriological loop to form secondary, tertiary and quaternary streak lines. These plates were incubated aerobically at 37^0 C for

24 hours and looked for evidence of growth. If there was no growth, the plates were further incubated for 48 hours and reported as no growth.

After isolation, Gram stain, and biochemical reactions were done according to the procedures described in Mackie and McCartney Practical microbiology and Koneman's textbook of Diagnostic Microbiology [10,11].

For Aerobic growth [10,11,12].
Days Procedure

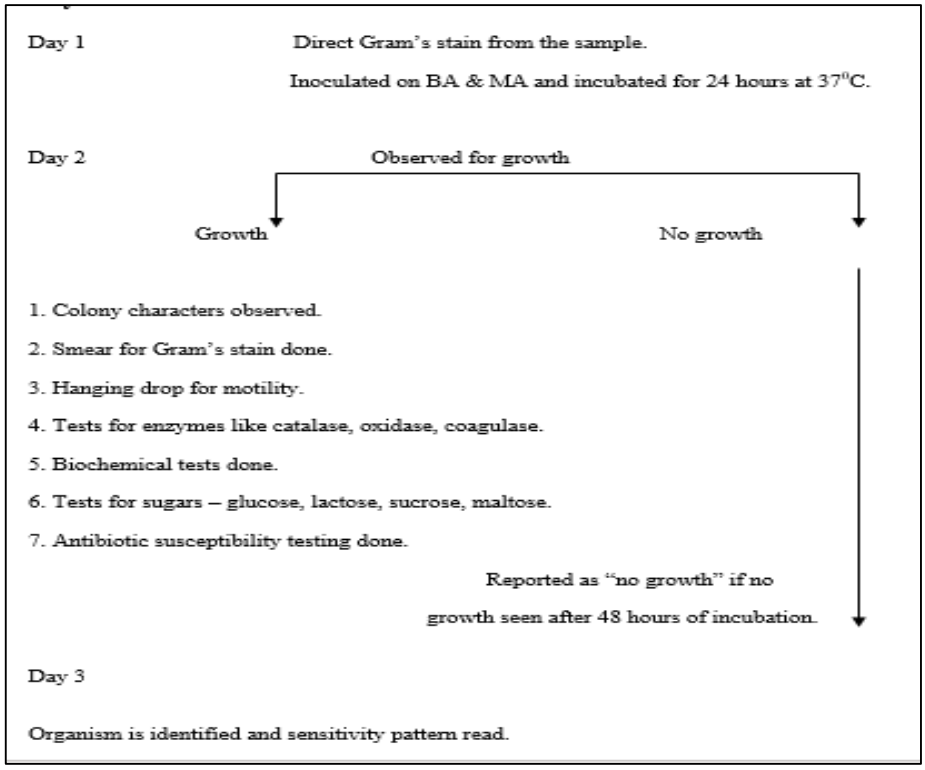

Fig-1: Gram stain, and biochemical reactions were done according to the procedures described in Mackie and McCartney Practical microbiology and Koneman's textbook of Diagnostic Microbiology [10,11].

Antibiotic Susceptibility [10,12].

Antibiotic susceptibility testing of the isolates was done on Mueller Hinton agar using Kirby- Bauer disc diffusion method. Bacterial suspension was prepared by inoculating few isolated colonies of similar morphology on to 4 - 5ml of peptone water and incubated at 37^0C for 2 - 4 hours. The turbidity of the growth was adjusted to 0.5 Mac Far land turbidity standards and lawn culture in made on the surface of the medium with sterile cotton swabs. The selected antibiotic discs were then placed aseptically on this media 1.5 cm apart using sterile forceps. The plates were incubated at 37^0c for 18-24 hours. The zone size recovered and reported as sensitive, intermediate or resistant. Commercially available Himedia discs were used.

Antibiotic discs used for gram positive organisms

1. Ampicillin (10 µg).
2. Azithromycin (15 µg).
3. Cotrimoxazole (25 µg).
4. Chloramphenicol (30 µg).
5. Gentamicin (10 µg)
6. Cefoxitin (30 µg).
7. Ofloxacin (5 µg).
8. Vancomycin (10 µg).
9. Cefuroxime (30 µg).
10. Ciprofloxacin (10 µg).

Antibiotic discs used for gram negative organisms:

1. Amoxyclav (30 µg)
2. Cotrimoxazole (25 µg)
3. Chloramphenicol (30 µg)
4. Gentamicin (10 µg)
5. Amikacin (30 µg)
6. Ofloxacin (5 µg)
7. Ceftriaxone (30 µg)
8. Piperacillin + Tazobactam (100/10 µg).
9. Ceftazidime (30 µg).
10. Ciprofloxacin (10 µg)

Results

A total of 93 clinically diagnosed cases of chronic suppurative otitis media attending ENT OPD and who those admitted in ENT IPD were studied. Observations made from the study are as follows.

I. Percentage of bacterial growth (culture positive): Out of 93 patients studied, 84 (90.3%) yielded bacterial growth, while 9 (9.68%) were sterile, out of 84 culture positive, 79 (84.95 %) were mono-bacterial and 5 (5.38 %) showed mixed bacterial growth.

II. Pattern of Organisms

Table-1: Number of 10 different bacterial species isolated from 89 isolates.

Organism	Frequency	Percentage (%)
1.*P. aeruginosa*	27	30.3
2. *S. aureus*	24	27.0
3. *K.pneumoniae*	8	9.0
4. *P.mirabilis*	10	11.2
5. *P.vulgaris*	1	1.1
6. *CONS*	5	5.6
7. *C. freundi*	5	5.6
8. *S. pyogenes*	2	2.2
9. *Acinetobacter spp*	2	2.2
10. *E. coli*	5	5.6
Total	89	100

A total number of 10 different bacterial species were isolated from 89 isolates.

Table 1 shows that the most common organism isolated was *Pseudomonas aeruginosa* (30.3%) followed by *Staphylococcus aureus* (27%), *Proteus mirablilis* (11.2%), *Klebsiella pneumoniae* (9 %), *Escherichia coli* (5.6%), *CONS* (5.6%), *Citrobacter freundi* (5.6%), *Acinetobacter spp* (2.2%), *Streptococcus pyogenes* (2.2%) *and Proteus vulgaris* (1.1%)

III. Antibiotic susceptibility pattern

A. Gram Negative Organisms

Table-2: Antibiotic susceptibility pattern.

Antibiotic Sensitivity %	*P. aeruginosa*	*P. mirabilis*	*P. vulgaris*	*K. pneumoniae*	*E. coli*	*C. freundi*	*Acinetobacter spp*	Total %
AMC	0	10	0	25	40	20	0	10.3
COT	0	70	100	62.5	80	100	50	66.1
C	51.9	60	100	50	100	100	100	63.8
GEN	66.7	40	100	37.5	40	100	0	56.9
AK	88.9	100	100	100	100	100	50	93.1
OF	100	80	100	100	60	100	100	93.1
CTR	0	100	100	50	60	100	0	53.1
PIT	88.9	90	100	100	100	100	50	89.84
CAZ	66.7	90	100	25	20	80	0	80.3
CIP	88.9	80	100	100	20	100	0	81

Among Gram negative isolates the most sensitive drug was amikacin and ofloxacin with 93.1% sensitivity, followed by piperacillin + tazobactam (88.9%) and ciprofloxacin (81%). Amoxyclav was the most resistant antibiotic, only 10.3% organisms were sensitive to it.

Gram Positive Organisms

Table-3: Antibiotic sensitivity against gram positive organisms.

Antibiotic Sensitivity %	*S. aureus %*	*CONS %*	*S. pyogenes %*	*Total %*
AMP	12.5	20	100	19.4
AZM	87.5	100	100	90.3
COT	83.3	80.7	0	77.4
C	95.8	100	100	96.3
GEN	91.7	100	100	93.5
CX	95.8	80	0	87.1
OF	100	100	100	100
VA	100	100	100	100
CXM	95.8	100	100	93.5
CIP	95.8	100	100	96.8

The most effective antibiotic against gram positive bacteria in the present study was found to be ofloxacin and vancomycin (both 100% sensitivity), followed by chloramphenicol (96%). Ampicillin was the most resistant antibiotic, only 19.4% organisms were sensitive to it.

Discussion

In the present study, an attempt was made to know the aerobic bacteriology of CSOM along with the antimicrobial susceptibility pattern of the isolates. The results are compared with the other studies and discussed as follows:

In the present study, 90.3% yielded bacterial growth and 9.68% were sterile (no growth). Among the culture positives, 84.95% showed monobacterial growth and 5.38% showed mixed bacterial growth.

Sterile cultures in the study can be attributed to non – bacterial cause, anaerobic growth, prior antibiotic therapy or presence of bacterial enzymes (lysozymes) which inhibit the growth of bacteria.

Poorey VK and Arati Iyer studied 100 patients with CSOM, pure growth was found in 82% of cases, mixed growth in 10% and no growth in 8% cases [5]. The present study also correlates with the findings of Hassan O et al, 107 patients with otitis media were studied, 93 yielded bacterial growth, while 14 showed no growth. Among those 93culture positive, 11 were mixed bacterial growth [9].A R. Shyamala and P.S. Reddy, 100 patients were studied, 93% showed culture positive, 7% specimen showed no growth [13]. Similar findings were reported by Srivastava et al also [2].

Pattern of Organisms: The most common organism isolated in the present study was *Pseudomonas aeruginosa* (30.3%) followed by *Staphylococcus aureus* (27%),

Proteus mirablilis (11.2%), *Klebsiella pneumoniae* (9%), *Escherichia coli* (5.6%), *CONS* (5.6%), *Citrobacter freundi* (5.6%), *Acinetobacter species* (2.2%), *Streptococcus pyogenes* (2.2%) *and Proteus vulgaris* (1.1%). Majority of the organisms were gram negative (65%).

The occurrence of *Pseudomonas aeruginosa* as prime offender can be attributed to:

a) Pseudomonas survives competition with other pathogens due to minimal nutritional requirement and its armamentarium of antibacterial products like pyocyanin and bacteriocin.
b) Vartiainen and Vartiainen postulated Pseudomonas has ability to carve a niche for itself in local infection through necrotizing activities of its extracellular enzymes.
c) In addition, the organism acts as an opportunistic pathogen, flourishes in the external auditory canal and may cause suppurative disease in contiguous sites.

R. Shyamala and P.S. Reddy conducted a study of bacteriological agents of CSOM in Nellore, Andhra Pradesh. 100 patients were studied, 93% showed culture positive, 7% specimen showed no growth. *P.aeruginosa* (40%) was the most common isolate followed by *S. aureus* (31%), *E. coli* (12%), *P. vulgaris* (5%) and *Klebsiella spp* (5%) [13].

Jagdish Kumar et al did a bacteriological study of CSOM by aerobic methods in a teaching hospital, 100 patients were studied. The most common aerobes were *P.aeruginosa* (54.8%), *S. aureus*, *Proteus spp* (35%), *Acinetobacter spp* and *diphtheroids*. Mixed culture the commonest combination was *P.aeruginosa* and *S. aureus* [14].

Shazia Parveen and Janardhan Rao. R studied 100 patients, the organisms isolated were *P. aeruginosa* (29.72%), *S. aureus* (21%), *CONS* (6.7%), *E. coli* (13%), *Proteus spp* (8%) and *Klebsiella spp* (6.7%) [15]. According to Ghulam Fatima et al, the common organisms were *P. aeruginosa (68%)* and *S. aureus (53%)* followed by *E. coli, Pneumococci, Enterococci, P. mirabilis and klebsiella spp* [16]. Moorthy et al did a microbiological study on CSOM; 90 patients were studied, the common organisms were *p. aeruginosa* (54%) and *S. aureus* (11.3%); followed by *P. mirabilis, K. pneumoniae, P. vulgaris, E. coli, S. saprophyticus and β-hemolytic streptococci* [17].

Asish J et al undertook a study of bacteriological and mycological profile of CSOM patients and their antibiotic sensitivity pattern; 120 patients attending ENT OPD were studied; the common bacterial isolated were *P. aeruginosa (33%), S. aureus (25%);P. mirabilis (20%), Enterobacter aerogenes (4%), s. pyogenes (3%), K. oxytoca, Acinetobacter species, S. pneumoniae* and *Providicia stuartii* [18]. S.K. Malkappa et al undertook a study of aerobic bacterial isolates and their antibiotic susceptibility pattern in CSOM; out of 130 samples studied, 126 (96.2%) were culture positive; Most common organisms isolated were *Pseudomonas aeruginosa* (45.24%) and *S. aureus* followed by *Klebsiella spp., E. coli, Proteus species*, Acinetobacter spp and *CONS* [7].

Arvind N, Pavan chand and Vishrutha KV did a study on microbiological profile of chronic suppurative otitis media. Out of 200 patients studied, of the patients were of the age group <10 years (35%) followed by 11-20 years (30%). Most common organisms isolated were *Pseudomonas aeruginosa* (41.5%) followed by *Staphylococcus* and members of the enterobacteriaceae family [19]. All these studies showed *P.aeruginosa* as the commonest causative agent for CSOM which is similar to the current study.

Staphylococcus aureus was the second most common organism with 27% in the present study. This compares with the studies of Varshney S et al [20], Hiremath et a [21] and Maji P.K et al [22].

Poorey V K and Arati Iyer undertook a study of bacterial flora of CSOM and its clinical significance, Aural swabs were taken from 100 patients. They found that the most common organisms causing CSOM as *Pseudomonas pyocyaneus (35.2%),* followed by *Klebsiella species (9.8%), Proteus species (9.8%), Escherichia coli (5.88%) Staphylococcus albus (4.9%) and Hemolytic streptococci (3.92%) cases. Proteus mirabilis* in the present study was third with 11.2% and in concordance with the Poorey VK and Arati Iyer's study [5].

Yousuf et al [23] and Prakash M et al [24] found the most common organism causing CSOM as *S. aureus*. Aminu A. Bakari et al reported *Klebsiella spp* as the most common causative organism causing CSOM based on their study [25]. Even these studies were comparable with the current study even though the prevalence of organisms were a little different.

So, after going through the various literatures, it's clear that the findings of the current study correlates well with most of the other studies on CSOM.

Antibiotic Susceptibility: The present study revealed that the most effective antibiotic against gram negative bacteria were ofloxacin and amikacin. 93% of the organisms showed sensitivity to both ofloxacin and amikacin. Amoxyclav was the most resistant antibiotic.

The most effective antibiotic against gram positive bacteria was ofloxacin. All the organisms were sensitive to it. Ampicillin showed maximum resistance.

The results of the present study are similar to the findings of Asish J et al where most of the organism were sensitive to gatifloxacin, cefoperazone - Sulbactam, and ceftriaxone; and resistant to ampicillin.[18].

The study on CSOM done by Sanjay Kumar et al showed the majority of the patients were sensitivity to cefoperazone and sulbactam (90.69%) followed by ofloxacin (79.06%) and ciprofloxacin (79%). Majority showed resistance to amoxicillin (95.35%) [26].

S.K Malkappa et al reported amikacin was the most effective antibiotic in their study. It was effective against maximum number of strains (82.37%), followed by ciprofloxacin (76.62%), cefoperazone (75.83%), gentamicin (71.4%) and ceftazidime (65.16%). Isolates (90%) showed resistance to amoxicillin.[7].

The study on CSOM by E. Meyer et al reported that the organisms isolated were susceptible to fluoroquinolones and aminoglycosides. Majority were resistant to amoxicillin. [27]. The study done by Moorthy et al showed most of the organisms sensitive to ciprofloxacin

The results of the present study is similar to the findings of S. Nikakhlagh et al [28]. Aminu A. Bakari et al reported that most (95.5%) of the organisms were sensitive to ofloxacin; but isolates of *P. aeruginosa* and *Klebsiella* showed resistance to ofloxacin.

Seventy five percent of gram negatives were sensitive to ceftazidime, cefotaxime, colistin and 61.36% of them were sensitive to gentamicin. Antimicrobial sensitivity was highest to quinolone antibiotics (57.9%) [25]. Jagdish Kumar et al, reported cephalosporins and fluoroquinolones (ofloxacin) showed maximum sensitivity [14].

According to Shanweel Ahmad's study, gentamicin had the highest sensitivity rate (88.3%), followed by chloramphenicol (72.4%) [29]. A.O. Okesola found that ciprofloxacin was the most effective antibiotic (77%) followed by ceftazidime (67.7%) [30].

The antibiotic susceptibility pattern of most of the studies in the past is correlating with the current study, which shows the general trend of antibiogram of bacteria causing CSOM is the same in many parts of the world.

Conclusion

The most common organism isolated was *P.aeruginosa* followed by *S. aureus*, *P. mirablilis*, *K. pneumoniae, E. coli*, *CONS*, *C. freundi*, *Acinetobacter spp, S. pyogenes, and P.vulgaris*. Majority of the organisms isolated were gram negative.

The most effective antibiotic against gram positive bacteria was found to be ofloxacin and Vancomycin. Among gram negative isolates the most sensitive drugs were amikacin and ofloxacin.

Chronic suppurative otitis media is a major health problem in many populations around the world and a significant cause of morbidity and mortality. It is particularly common in developing countries. It is a major cause of hearing impairment and the effect is major concern particularly in children, because it may have long-term effects on early communication language development, auditory processing, psychosocial and cognitive development and educational progress. The aim of this study was to identify potential causative agents associated with CSOM especially in an environment where antibiotics are commonly abused.

Based on the findings from this study, it is therefore recommended that treatment of ear infection is better done when the causative agents as well as the drug sensitivity patterns are known and properly administered. This will enhance better treatment, reduce the incidence of drug resistance and reduce the burden of the infection on the patients and in the long term, it may also reduce the cost of treatment.

What the study adds to the existing knowledge?

The most common organisms causing CSOM were found to be *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The present study revealed that the most effective antibiotic against gram negative bacteria were ofloxacin and amikacin. The most effective antibiotic against gram positive bacteria were ofloxacin and vancomycin.

This will help clinicians to choose the right antibiotic for empirical therapy in CSOM suspected cases, awaiting culture results. This can avoid complications of CSOM, particularly in children, and reduce the chance of antibiotic resistance.

Author's contribution

Dr. Kiran Gopal: Design of study protocol, ensured proper methodology, review of literature & manuscript.

Dr. Shivakumar S Solabannavar: Guided the research work & manuscript editing.

Dr. Mohammed Riyas: Collection of data & Statistical analysis

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Conflict of interest: None declared
Ethical Approval: This study was approved by the Institutional Ethics Committee

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