Bacteriological Profile, Drug Resistance Pattern and its Molecular Characterisation Among Patients with Chronic Suppurative Otitis Media at a Tertiary Care Hospital in South India

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        Millions of people worldwide suffer from chronic suppurative otitis media (CSOM), a recurrent infection of the ear that is particularly common in low-resource environments. Although there are several etiological factors, bacterial infection is a significant one. The two most frequently isolated bacteria are *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Due to the emergence and spread of bacterial resistance to several antimicrobial agents and also their ability to form biofilms, treatment and recovery are made more challenging. Our aim was to identify the clinico-bacteriological profile, their antimicrobial susceptibility pattern and a molecular method to identify the antibiotic resistance genes of the most common isolates in this study. Following approval from the Institutional Human Ethics Committee (IHEC) and patient informed consent, we obtained ear discharge samples from 100 CSOM patients over a two-year period. The isolates were recognized by conventional microbiological methods. Polymerase chain reaction (PCR) was used for molecular characterisation in order to identify genes linked to antibiotic resistance (e.g., efflux pump-related genes associated with *P. aeruginosa* and mecA for *S. aureus*). Of the 100 patient samples, 84 samples showed positive results for culture; 58% of the Gram-positive cocci were *S. aureus* and 42% of Gram-negative bacilli were *P. aeruginosa*. Methicillin resistance in *S. aureus* is indicated by the presence of the mecA gene (43%) and carbapenem resistance in *P. aeruginosa* is indicated by the presence of the blaVIM gene (22%), according to genotypic study. Our work provides insight with regard to the genetic makeup of *P. aeruginosa* and *S. aureus* in CSOM patients, which demonstrates that these bacteria have several resistance genes that enable them to withstand antimicrobial therapy.

Keywords: Chronic suppurative otitis media (CSOM); Coagulase negative *Staphylococcus* (CoNS); multidrug resistant (MDR); Methicillin resistant *Staphylococcus aureus* (MRSA); *Pseudomonas aeruginosa; Staphylococcus aureus*.

"Chronic suppurative otitis media (CSOM) is characterised by a persistent infection of the middle ear cleft, which includes the middle ear, mastoid, eustachian tube, as well as a perforated tympanic membrane and otorrhea, that lasts for two weeks or more". It is sometimes referred to as chronic tympanomastoiditis, chronic otomastoiditis, and chronic active mucosal otitis media.[1] This typically affects children and develops as a side effect of acute otitis media.[2] In about half of the patients, suppurative discharge and hearing loss are the most prevalent presentations.[3] It is

more prevalent in developing countries, especially among lower socioeconomic classes with limited health care, overcrowding, poor hygiene, and malnutrition which significantly lowers one's quality of life.[4,5] Risk factors include prior upper respiratory tract infections, traumatic tympanic rupture, and recurrent episodes of acute otitis media.[6]

Globally, the CSOM prevalence ranges from 1 to 46%. It is more prevalent in South-east Asian countries, Africa, the Western Pacific.[7] CSOM is uncommon in the United states, Australia, Middle East and Europe[8]. It is estimated that there are between 65 and 300 million instances globally, and that 60% of these cases result in substantial hearing impairment.[9] It has been stated that CSOM problems result in 28,000 fatalities annually worldwide. The annual cost of otitis media and its consequences, such as CSOM[10], is estimated to be approximately $5 billion in the United States. Anaerobes, aerobes, and fungi are considered potential pathogens in CSOM, though their reported profiles and frequencies vary depending on the geography, age, and presence of complications such as cholesteatoma.[11] Aerobic bacteria such as *Escherichia coli*, *Proteus* species, *Klebsiella pneumoniae* and *Streptococcus pyogenes* are commonly isolated after *Pseudomonas aeruginosa and Staphylococcus aureus,* while *Bacteroides, Peptostreptococcus* and *Propionibacterium* are the most common anaerobes. As with other bacterial infections, multidrug-resistant (MDR) bacteria are a growing concern in CSOM.[12] A significant incidence of multidrug resistance against amoxycillin, amoxycillin/clavulanic acid, ampicillin, cephalosporins, macrolides, co-trimoxazole, quinolones has resulted in adverse treatment outcomes in recent years.[13]

In addition to preventing complications from CSOM, early identification of the causative bacteria and their susceptibility is essential for a satisfactory clinical recovery. Appropriate antimicrobial therapy is employed to eliminate the bacterial agents responsible for otitis media; nevertheless, the majority of microorganisms are developing resistance to antibiotics.[14]

The bacteria causing CSOM are becoming more MDR as a result of antibiotic overuse and misuse causing this infection to spread quickly in underdeveloped nations[15], which necessitates routine surveillance of the microbiological and susceptibility profiles.[16] Additionally, knowing the range of bacteria and how susceptible they are to antibiotics will help us understand CSOM better and will be essential for successful empirical treatment.[14] Accordingly, the current study identified the clinico-bacteriological profile of CSOM, examined their susceptibility pattern of different antibiotics and used a molecular technique to identify the antibiotic resistance genes of the most common isolates.

MATERIALS AND METHODS

Study area

The study consisted of 100 CSOM patients attending ENT department at a tertiary care facility in Chennai, South India. The ear discharge samples were collected using sterile swabs and transported to the Department of Microbiology, Central Laboratory.

Inclusion criteria

a) 100 clinically diagnosed CSOM patients of different age groups. b) active ear discharge for at least 3 months c) discharge from one/both ears were included in the study.

Exclusion criteria

a) active ear discharge of less than 3 months duration b) patients who were recently treated for CSOM c) patients with ear discharge and intact tympanic membrane d) samples which had growth other than bacteria e) patients with congenital ear, obstructed middle ear, malignancy, prior ear surgery were excluded from the study.

Study design

A cross-sectional study was conducted from August 2022 to July 2024 after obtaining approval from Institutional Human Ethics Committee (IHEC) (Ref. no. 002/SBMCH/IHEC/2022/1806) and informed consent from all the participants. The samples were subjected to microbiological analysis and genotypic detection of the predominant bacteria by PCR.

Microbiological analysis

Two ear swabs from each patient were aseptically taken after a thorough inspection of the ear using an otoscope. One ear swab was subjected to direct microscopy and the other was used to inoculate on nutrient agar, MacConkey agar and blood agar. The plates were incubated at

37! for 18-24 hrs and identification of organisms was done based on standard bacteriological techniques.[17] Muller-Hinton agar was used for antimicrobial susceptibility testing, and the results were interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) recommendations 2022.[18]

Antibiotics Tested for *Staphylococci*

Penicillin (10µg), cefoxitin (30µg), ciprofloxacin (5µg), levofloxacin (5µg), gentamicin (10µg), erythromycin (15µg), clindamycin (2µg), linezolid (15µg), tetracycline (30µg), co-trimoxazole (25µg), vancomycin (30µg), teicoplanin (30µg)

Antibiotics Tested for other Gram Positive Cocci

penicillin (10µg), ampicillin (10µg), chloramphenicol (30µg), erythromycin (15µg), clindamycin (2µg), tetracycline (30µg), levofloxacin (5µg), high level gentamicin (120µg), linezolid (15µg), vancomycin (30µg), cefepime (30µg), cefotaxime (30µg), ceftriaxone (30µg)

Antibiotics Tested for *Pseudomonas* Species

amikacin (30µg), gentamicin (10µg), ciprofloxacin (5µg), levofloxacin (5µg), piperacillin-tazobactam (100/10µg), ceftazidime (30µg), cefepime (30µg), aztreonam (30µg), meropenem (10µg), cefoperazone-sulbactam (75/30µg)

Antibiotics Tested for other Gram Negative Bacilli

ampicillin (10ìg), amoxycillin-clavulanic acid (30µg), levofloxacin (5µg), amikacin (30µg), gentamicin (10µg), cefepime (30µg), ceftriaxone (30µg), cefotaxime (30µg), ceftazidime (30µg), cefazolin (30µg), piperacillin-tazobactam (100/10µg), meropenem (10µg), co-trimoxazole (25µg), aztreonam(30µg)Organisms resistant to more than 3 class of antibiotics was considered as multidrug resistant (MDR). Phenotypic tests for extended spectrum beta-lactamase (ESBL) & methicillin-resistant *Staphylococci* were performed[18]. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were utilized as controls. The media, reagents, antibiotic discs, ATCC strains were purchased from HiMedia, Mumbai, India. For additional validation of the pathogenic organism and its antimicrobial susceptibility, the Vitek-2 compact automated system (Vitek-2 GN card and Vitek-2 GP card, BioMérieux Inc., Durham, NC) was utilized.

Polymerase chain reaction (PCR) was done to find resistant genes of predominant bacteria.

Genotypic detection of predominant bacteria[19,20]

PCR amplification was done for all the MDR *Pseudomonas* and methicillin resistant *S. aureus* isolates to detect the genes encoding resistance bla_{VIM} genes and *mecA* genes respectively. Individual colonies were picked up from the quadrant streak plates and were amplified by 16S rRNA primer. A PCR template was created by dissolving each colony in 23.8 µl of distilled water. Every colony underwent PCR, and the amplified PCR products were examined in a 1% agarose gel to ensure their quality and quantity. Primer details: bla_{VIM} F:GATGGTGTTTGGTCGCATA R: CGAATGCGCAGCACCAG *mecA* F-AAAATCGATGGTAAAGGTTGGC) R: AGTTCTGCAGTACCGGATTTTGC3')

Statistical analysis

For data entry, Microsoft Excel was used. The data were analysed and P-value was calculated using IBM Corp.'s Armonk, NY SPSS, Version 25.0. Tables showed the frequency and percentage representations of the descriptive statistics.

RESULTS

In our study, out of 100 CSOM patient samples, 59% were from male patients and 41% were from female patients. Table 1 shows the distribution of samples among various age groups.

Unilateral involvement of the ear was seen in 95% of the patients (right ear-56 and left ear-39) and bilateral involvement was seen in 5% of the patients. Out of the 100 CSOM patients, 84 patients yielded growth in culture. Among the culture positive patients, 17 (20%) had polymicrobial growth and 67 (80%) had monomicrobial growth which yielded a total of 101 isolates. Out of 101 isolates, 53 (52%) were Gram-negative bacilli and 48 (48%) were Gram-positive cocci. Table 2 and table 3 shows the prevalence of Gram-negative bacilli and Gram-positive cocci among CSOM patients, respectively.

The prevalence of antimicrobial resistance to the most commonly used antibiotics are depicted in figures 1 and 2.

It was found that 32% of *P. aeruginosa*, 46% of *K. pneumoniae*, 40% of *E. coli*, 20% of *P. vulgaris* were extended spectrum beta-lactamases

(ESBL) producers. The prevalence of methicillin resistant *S. aureus* (MRSA) and methicillin resistant CoNS (MRCoNS) by phenotypic detection method were found to be 43% and 57% respectively.

The prevalence of bla_{VIM} gene among MDR *P. aeruginosa* isolated from CSOM patients by PCR is shown in table 4.

Figure 3 shows the amplification of bla_{VIM} gene by PCR in 2 selected *P.aeruginosa* isolates

The table 5 describes the prevalence of *mecA* gene among *Staphylococci* isolated from CSOM patients.

Figure 4 shows the amplification of mecA gene by PCR in 5 selected *S.aureus* isolates

DISCUSSION

CSOM is a chronic disease that affects people of all ages and has a low recovery rate. The prevalence of ear infections varies among different populations. The WHO reports that the prevalence of CSOM in India is 7.8%, placing the country among the highest in the world and necessitating prompt attention to address a serious public health issue.[5] In order to prevent major complications

from CSOM, early microbiological identification is essential for both timely and efficient treatment.

The current study reported male and female prevalence were 59% and 41% respectively. A recent study reported that about 58% infected were males, 42% were females.[21] A number of studies have revealed a male predominance, which may be related to their active lifestyles and hobbies like diving and swimming.[22] Recurrent infections and intermittent ear discharge can result from swimming-induced middle ear soiling. The increase in health awareness among women may be the reason for their lesser preponderance, since they tend to seek care earlier.

In our study, the predominant age was 40-60 years (27%) and 20-40 years (26%) followed by 11-20 years (19%), <10 years (18%) and >60 years (10%). A similar study reported 40% infected were children and 60% were adults.[23] Another recent study found that the predominant age group was 41-65 years (54%).[22] The fact that eustachian tubes in children are shorter, thinner, and more horizontal than those of adults may be the cause of the high occurrence of CSOM in children.[24] Additionally, there is an increased risk of upper respiratory

Table 1. Distribution of samples among various age groups

Age Group	Frequency (n)	%
<10 years	18	18
11-20	19	19
20-40	26	26
40-60	27	27
>60	10	10
Total	100	100
Mean age	44.8 years	

Table 2. Prevalence of Gram-negative bacilli among CSOM patients

GNB isolates	Frequency
Pseudomonas aeruginosa	22 (42)
Klebsiella pneumoniae	13 (25)
E.coli	10 (19)
Proteus vulgaris	5 (9)
Proteus mirabilis	2 (4)
Enterobacter species	1 (2)
Total	53 (100)

Table 3. Prevalence of Gram-positive cocci among CSOM patients

GPC isolates	Frequency
Staphylococcus aureus	28 (58)
CoNS	14 (29)
Streptococcus pyogenes	2 (4)
S. pneumoniae	1 (2)
other *Streptococcus* spp.	1 (2)
Enterococcus faecalis	2 (4)
Total	48 (100)

Table 4. PCR identification of bla_{VIM} gene among MDR *P. aeruginosa*

bla_{VIM}	No. of MDR *P. aeruginosa* isolates (%)
Positive	2 (22%)
Negative	7 (78%)
Total	9 (100%)

tract infections (URTIs), which can lead to ear infections, in this age range.

The current study reported culture positivity for 84% samples. Among them 17% were polymicrobial and 67% were monomicrobial. A range of 84% to 91.18% has been recorded in several Indian researches on the culture positivity rate. A recent study reported 73% of the samples were positive for culture.[25] Another study reported higher culture positive reports (94%).[26] In contrast, a study reported only 69.2% culture positive.[24] Studies reported with less percentage of culture positive may be due to patient's prior treatment with broad spectrum antibiotics before obtaining the sample.

Among 48 Gram positive cocci isolates,
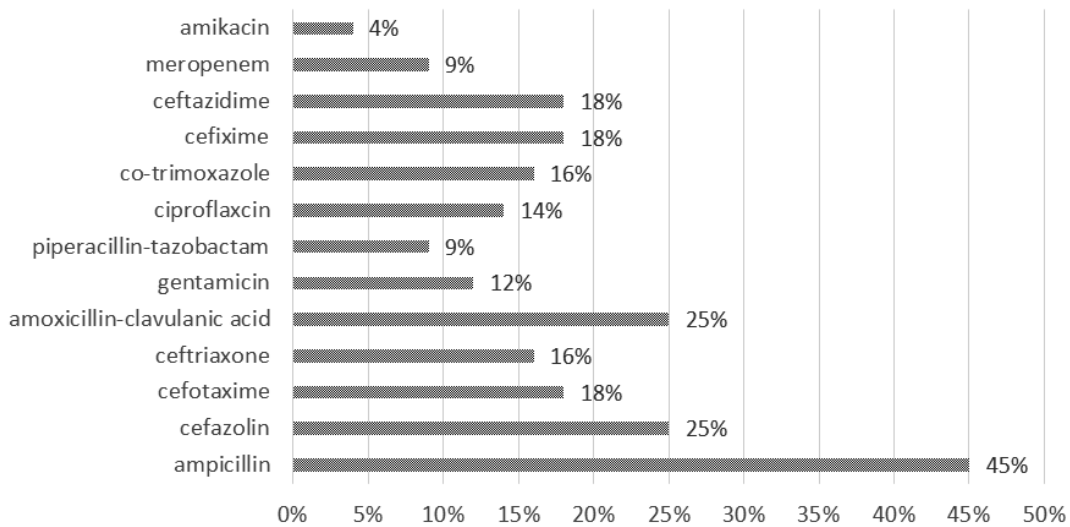
Antimicrobial resistance pattern among GNB

Fig. 1. Antimicrobial resistance pattern among Gram-negative bacilli

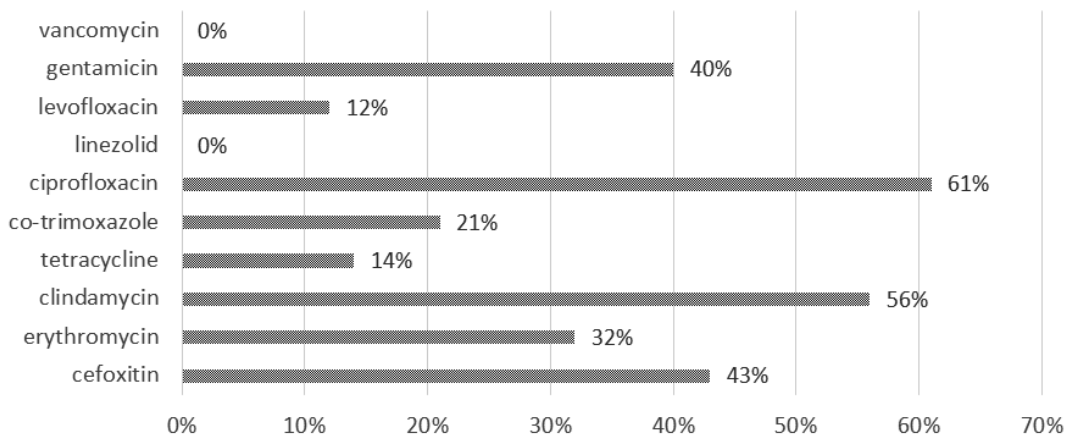## Antimicrobial resistance pattern of Staphylococcus aureus

Fig. 2. Antimicrobial resistance pattern among *S. aureus*

predominant species was *S. aureus* (58%) followed by CoNS (29%), *Streptococcus* spp. (8%), *Enterococcus* spp. (4%). Out of 53 GNB, *P. aeruginosa* (42%) was the predominant bacilli followed by *K. pneumoniae* (25%), *E. coli* (19%), *P. vulgaris* (9%), *P. mirabilis* (4%) and *Enterobacter* spp. (2%). According to a research, *S. aureus* (30.35%)[27] and *P. aeruginosa* (44.64%) were the two most common bacteria which was consistent with our study. Predominance of Gram-negative bacilli (59.74%) was reported

in a related study where *P. aeruginosa* had the highest incidence (45.5%), followed by *S. aureus* (37.7%).[28] A higher rate of *P. aeruginosa* has its own consequences because *Pseudomonas* is a major pathogen in nosocomial infection and can spread resistance-carrying plasmid to other species. According to another study, *S. aureus* was predominant followed by *Pseudomonas* species.[6] *S. aureus* is a common cause of middle ear infections, and its high resistance strain carriage in the upper respiratory tract and external auditory canal can

Table 5. Prevalence of *mecA* gene among *Staphylococci*

Staphylococci	Total (n=20)	*mecA* positive	*mecA* negative	P Value	OR	95% CI
MRSA	12	12 (100%)	0	0.068	1.333	0.89-1.98
MRCoNS	8	6 (75%)	2 (25%)			

Fig. 3. L1 – 1000Bp ladder, L2 – negative control for 710bp *bla*$_{VIM}$
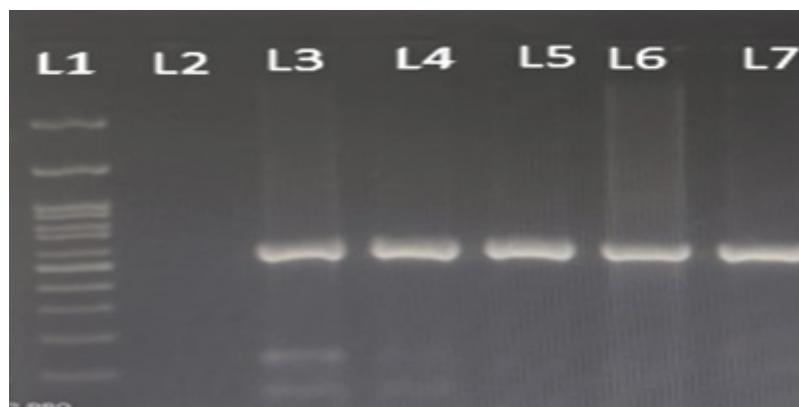L3 to L4 – *bla*$_{VIM}$ amplification positive strains

Fig. 4. L1 – 100 Bp ladder, L2 – negative control for *mecA*
L3 to L7 – *mecA* 520bp positive strains

be ascribed to this fact. *Pseudomonas* species are primarily considered as secondary invaders from the external auditory canal that enter the middle ear through a tympanic membrane defect brought on by an acute episode of otitis media. When the resistance is low, bacteria like *Proteus* spp., *Klebsiella* spp. and *E. coli* turn into opportunistic pathogens in the middle ear.

In this study, among *S. aureus* alarming resistance to ciprofloxacin (61%) was observed, which may have resulted from irrational use, insufficient dosage, or over-the-counter availability. On the other hand, the lesser resistance to levofloxacin (12%) was seen. MRSA which is 100% susceptible to vancomycin and linezolid was identified which was in line with a similar study.[6] Gram-negative bacteria in our investigation were more susceptible to amikacin, piperacillin-tazobactam and meropenem. Maximum resistance to ampicillin and cephalosporins were observed. These concur with the similar research conducted in CSOM patients.[29]

In the present study we reported among 12 MRSA, all the isolates showed positive for *mecA* gene amplification and among 8 CoNS isolated 6 (75%) were positive for *mecA* gene. In a study among MRSA isolates from ear swab, 50% of the isolates recovered *mecA* gene and 66.6% of the MRCoNS were positive for *mecA* gene.[30] Among the predominant Gram-negative bacilli in the current study, bla_{VIM} gene by PCR amplification of MDR *Pseudomonas* strains were reported. Among 9 isolates of MDR *P. aeruginosa*, 2(22%) were positive for bla_{VIM} gene. Research has revealed that MDR *P. aeruginosa* clones are linked to metallobeta-lactamases (MBL) genes, primarily VIM and IMP, which can be acquired through horizontal gene transfer or chromosomal mutations.[31] Another study reported 40% carbapenem resistant *P. aeruginosa* (CRPA) isolates harbored the bla_{VIM} genes respectively.[32] In contrast to our research, a study reported that 39% of the isolates of *P. aeruginosa* were MDR and that no isolate had bla_{VIM} genes found in it.[33] The aforementioned research shown that the microbial and their genotypic profile varies depending on patient population and geographical distribution between different places. Therefore, it strongly suggests that the microbiological profile in every geographic area be regularly examined and updated.

Limitations of this Study

In our study, on comparing the prevalence of *mecA* gene among *Staphylococci,* the p-value was 0.068 (nearly statistically significant) because of the less number of MRSA and MRCoNS isolated. Also, other causes of CSOM such as anaerobes, fungi were not included in this study.

Hence, further research is encouraged to get beyond these limitations.

CONCLUSION

In this study we determined that *S. aureus* and *P. aeruginosa* were the primary etiological agents of CSOM. Understanding the bacterial profile and their antimicrobial susceptibility pattern are crucial for patient therapy as it determines when to start antimicrobial therapy, which lowers treatment costs. The strains of *P. aeruginosa* that produced bla_{VIM} were the predominant ones in our study. Threatening therapeutic options, the advent of carbapenem resistance highlights the necessity for continued epidemiology and antimicrobial susceptibility research as well as long-term monitoring of antibiotic prescription practices. Hospital infection control teams should be concerned about the ongoing existence of these harmful organisms and how they may spread both within and between hospitals. The most successful way to treat CSOM is to choose topical or systemic antibiotics carefully, based on the culture and sensitivity reports, while maintaining dry ears to avoid drug resistance, unintended antibiotic delivery, and other problems. Antibiotic therapy is not always necessary for otitis media, but it should be used with caution when prescribing medication in cases of middle ear infection to avoid the development and dissemination of resistant bacterial strains in hospital and community settings.

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Conflicts of Interest

The authors do not have any conflict of interest

Data Availability Statement

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement

This cross-sectional study was conducted after obtaining approval from Institutional Human Ethics Committee (IHEC), Sree Balaji Medical College and Hospital (SBMCH), Bharath Institute of Higher Education and Research (BIHER), Chennai-600044 (Ref. no. 002/SBMCH/IHEC/2022/1806)

Informed Consent Statement

Informed consent was obtained from all the participants in the study and it conforms to the standards currently applied in our country. The privacy rights of human subjects has been observed in our study.

Author Contributions

Each author mentioned has significantly and directly contributed intellectually to the project and has given their approval for its publication; Chitralekha Saikumar: Visualization, Resources, Supervision, Project Administration; Punithavathi Velmurugan: Data Collection, Analysis, Writing – Original Draft; Aishwarya J Ramalingam: Conceptualization, Methodology, Writing –Review & Editing.

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