



Research Article

Microbiological Isolates of Chronic Suppurative Otitis Media at the University Teaching Hospital and Beit Cure Hospital in Lusaka, Zambia

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Abstract: *Background:* Chronic Suppurative Otitis Media (CSOM) is a common cause of hearing loss and many complications such as meningitis. Many approaches to the treatment of CSOM have been unsatisfactory because CSOM microbiological isolates, as well as their sensitivity patterns, vary from place to place. This study sought to determine the pattern of microbiological isolates of CSOM and the demographic characteristics of patients with CSOM at the University Teaching Hospital, (UTH) and Beit Cure Hospital (BCH) in Lusaka, Zambia. *Materials and Methods:* The study was a hospital based Cross-sectional descriptive study conducted at the ENT outpatient clinics of UTH and BCH in Lusaka, Zambia. 100 CSOM patients were included in the study. Quantitative data on the participants' demographic details and clinical features were obtained using structured questionnaires. The middle ear discharge was aseptically collected using a sterile cotton swab. In the laboratory, samples were inoculated on agar media to isolate microorganisms and antibiotic susceptibility testing was done using Kirby-Bauer method as per CLSI guidelines. *Results:* From the findings, out of the 100 CSOM patients studied, 33(33%) were children below 18yrs and 67(67%) were adults. 59(59%) of the patients had unilateral CSOM while 41 had bilateral CSOM which gave a total of 141 ears that were analyzed. 119(84.4%) had pure cultures, 20(14.2%) had mixed cultures and 2(1.4%) had no growth. Of the 169 microbiological isolates, the most frequent isolates were *Proteus mirabilis* 49(29.0%), *Pseudomonas aeruginosa*, 32(18.9%), coagulase negative *Staphylococcus* 18(10.7%) and *klebsiella pneumoniae* 17(10.1%). High sensitivity rates were revealed to Gentamycin (64-100%), meropenem (68-100%), ceftazidime (85-100%), ceftriaxone (64-80%), and ciprofloxacin (66-88%). High resistance rates were recorded to Amoxicillin-clavulanate (as high as 100%), ampicillin (as high as 100%), tetracycline (as high as 91.2%) and cotrimoxazole (as high as 100%) and penicillin (as high as 100%). *Conclusion:* The study concluded that *Proteus mirabilis* was the most dominant microbiological isolate followed by *Pseudomonas aeruginosa*. The isolated microorganisms had high susceptibility rates to gentamycin, meropenem, ceftazidime, ceftriaxone and ciprofloxacin. There were high resistance rates to amoxicillin-clavulanate, ampicillin, tetracycline, cotrimoxazole and penicillin.

Keywords: Chronic Suppurative Otitis Media, Microbiological Isolates, Sensitivity, Resistance

1. Introduction

Chronic suppurative otitis media (CSOM) is chronic inflammation of the middle ear cleft (Eustachian tube, middle ear, and mastoid cavity) which presents with recurrent ear discharge or otorrhea through a tympanic perforation for two weeks or more.¹ CSOM commonly occurs during the first 6 years of a child's life, with a peak around 2 years.² According to WHO, about 330 million individuals, globally, have CSOM. In Africa, over 2.4 million people have CSOM, accounting for almost 4% of the global CSOM burden.¹

The pathogenesis of CSOM is multifactorial with factors such as Eustachian tube (ET) dysfunction, genetic predisposition, and environmental factors playing a role.³ A dysfunctional and structurally immature ET is the most important factor in the pathogenesis of otitis media (OM).⁴ Infants and young children are especially at risk for reflux (containing bacteria and viruses) into the middle ear from nasopharynx via the ET because their ET is short, horizontal, and 'floppy'.³ The bacteria and viruses in the middle ear elicit an inflammatory response causing an acute infection. CSOM is initiated by an episode of acute infection of the middle ear that fails to resolve and result in a permanent TM perforation.⁴

The common causative organisms of CSOM include aerobic bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Haemophilus influenza*, *Staphylococcus aureus* and *Klebsiella* species.^{5,6} Anaerobic bacteria identified as CSOM causative organisms include *Bacteroides* and *Fusobacterium* species.⁷ *Aspergillus* and *Candida* species are common fungal isolates of CSOM.^{1,8} The sensitivity pattern of CSOM causative organisms varies generally from place to place due to differences in climatic conditions and manner of antibiotic use.⁹

Apart from being a cause of complications such as facial palsy, mastoiditis, brain abscess and labyrinthitis, CSOM is a major cause of acquired hearing impairment, especially in developing countries.¹ Educational, vocational and social problems are some of the problems that stem from hearing impairment. Other problems include impaired speech and language development, poor academic performance and poor social interaction.¹

2. Materials and Methods

This study was a hospital based cross-sectional descriptive study conducted in the ENT outpatient clinics at the university Teaching Hospital (UTH) and Beit Cure Hospital (BCH) which are situated in Lusaka, Zambia. Ethical approval was obtained from the KNH-UON Ethics Research Committee in Nairobi, Kenya; ERES (Excellence in Research and Science) Converge Ethical and Research Committee in Zambia; and from the aforementioned Hospitals. The sample size of 103 CSOM patients inclusive of 10% attrition was determined by the Yamane formula (1967:886)¹⁰ with a precision error of 5%. The study group was as described under the section 2.1 below. The collection of materials for laboratory analysis and susceptibility

tests have been described in section 2.2 and 2.3 respectively. Quality control was a continuous process throughout the study to maximize validity and reliability of the findings of the study. Measures that were put in place included the use of trained health professionals; aseptic techniques in collecting specimen; internal quality assessment of the procedures in the laboratory.

2.1. Study Group

The respondents were patients of all age groups who had actively draining CSOM (using WHO CSOM definition) and were attending the outpatient ENT clinics at UTH or BCH. They were patients who were not on any antibacterial and anti-fungal medication (at least in the last 2 weeks before participating in the study) and did not have any known immunosuppressive diseases. These were patients who consented or for whom a legal guardian had consented to participate in the study.

2.2. Bacterial Isolation

Using an aseptic technique (outlined below) pus discharge from the participants discharging ears was collected by the principal investigator within the ENT clinic on the first day of contact before any topical or systemic antibiotics or anti-fungal medication was started. Using sterile gloves (after washing hands with soap) under direct visualization with good lighting, and under microscopy for the majority of the patients, a sterile swab was passed through a sterile aural speculum placed in the EAC (to avoid contamination from the skin of the auditory canal) and then advanced to the middle ear or the inner two-thirds of the EAC to collect pus specimen. The sample obtained was put in a swab transport tube that contained a transport media and then labeled with a unique patient identifier and sent to the UTH microbiology laboratory. In the laboratory, by the laboratory technician, the specimens collected were inoculated on sheep Blood Agar, MacConkey's media, and chocolate agar media to culture aerobic bacteria. Anaerobic blood agar incubated in an anaerobic jar was used to culture anaerobic bacteria. Fungi were cultured on Sabouraud's dextrose agar. The culture plates were incubated at 37°C for 24-48 hours. Owing to the fact that anaerobes grow slowly compared to aerobes, anaerobic culture plates were incubated for up to 7 days to allow for anaerobic bacterial growth. Isolates from the culture plates were identified Using gram staining, colony morphology, catalase, coagulase, oxidase and biochemical strips. Lactophenol cotton blue was used for final identification of fungal growth.

2.3. Antibiotic Susceptibility Testing

Antimicrobial susceptibility tests were done on Mueller-Hinton agar using disk diffusion method as described by Kirby-Bauer. The antimicrobial agents tested were: tetracycline (30µg), chloramphenicol (30µg), gentamicin (10µg), ciprofloxacin (5µg), cotrimoxazole (25µg), ceftriaxone (30µg) and amoxicillin-clavulanate (10µg), meropenem, oxacillin, ceftazidime, cefoxitin, cefotaxime,

ampicillin, and penicillin. Susceptibility data were interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2015) by the microbiologist.

2.4. Statistical Analysis

Descriptive statistics were done using percentages of proportions, mean and standard deviation; Chi-square tests were done to establish relationships between variables using SPSS (Statistical Package for the Social Sciences) version 21.

3. Findings

3.1. Demographic Characteristics and Clinical Findings of CSOM Patients

A total of 100 CSOM patients were studied. The age range of

the study patients was 6 months to 68 years. Of the patients studied, 33(33%) were children under the age of 18, while 67(67%) were adults. The mean age was 24.5(\pm 18.0) years. The majority of the patients with CSOM in this study, 81(81%), resided in peri-urban areas which are associated with a low socioeconomic status. Other demographic characteristics of the respondents are as shown in Table 1.

Of the patients studied 59(59%) had unilateral CSOM while 41 had bilateral CSOM which gave a total of 141 ears that were analyzed. Central perforation was the most common type of tympanic membrane perforation seen in 119(84.4%) ears, of which 43(30.5%) were subtotal. Attic perforations were seen in 2 (1.4%) ears and marginal perforations in 9 (6.4%) ears as shown in Table 1. Granulation tissue was present in 20(14.2%) and cholesteatoma in 17(12.1%).

Table 1. Social-Demographic characteristics and clinical findings

Social-Demographic characteristics			
Characteristic	Category	Frequency	Percent
Gender	Male	57	57.0
	Female	43	43.0
Respondent	Children	33	33.0
	Adults	67	67.0
Residence	Urban	19	19.0
	Peri-urban	81	81.0
	Tertiary	23	34.3
Level of education of adult patients	Primary school	15	22.4
	Junior secondary school	15	22.4
	High school	12	17.9
	Illiterate	2	3.0
Occupation of patient	Unemployed	34	34.0
	Employed	29	29.0
	Casual worker	4	4.0
Occupation of parent	Employed	21	21.0
	Unemployed	6	6.0
	Casual worker	6	6.0
Size of household population	<6	55	55.0
	6-10	40	40.0
	>10	5	5.0
Type of cooking fuel used	Charcoal	63	63.0
	Electricity	35	35.0
	Firewood	2	2.0
Household member smokes	Does not smoke	83	83.0
	Smokes	17	17.0
Age groups	<=5	19	19.0
	6-10	7	7.0
	11-15	7	7.0
	16-20	15	15.0
	21-25	7	7.0
	26-30	13	13.0
	31-35	6	6.0
	36-40	8	8.0
	41-45	5	5.0
	46-50	5	5.0
	51-55	2	2.0
	61-65	1	1.0
Examination findings	66-70	5	5.0
	Unilateral	59	59.0
Presence of Otorrhea	Bilateral	41	41.0
	Central	119	84.4
TM perforation	Subtotal	43	30.5
	Total	11	7.8
	Marginal	9	6.4

Social-Demographic characteristics			
Characteristic	Category	Frequency	Percent
	Attic	2	1.4

3.2. Laboratory Findings

Of the 141 specimens analyzed, 103(73.0%) had gram negative rods, 22(15.6%) had gram-positive cocci, 9(6.4%) had gram-negative cocci, and 8(5.7%) had fungal elements. Pure cultures were 119(84.4%) and mixed cultures were 20(14.2%). 2(1.4%) specimens had no growth. Of the 169 microbiological isolates obtained, the most dominant was *Proteus mirabilis*, 49(29.0%). Other isolates are as shown in Table 2 below.

Table 2. Microbiological isolates.

Microbiological Isolates	Oxygen requirement		Percent
Gram -ve Bacteria	Facultative anaerobes (n)	Aerobes (n)	
<i>Proteus mirabilis</i>	49		29.0%
<i>Proteus vulgaris</i>	3		1.8%
<i>Pseudomonas aeruginosa</i>		32	18.9%
<i>Pseudomonas spp not aeruginosa</i>		17	10.1%
<i>Klebsiella pneumoniae</i>	17		10.1%
<i>Klebsiella oxyntica</i>	2		1.2%
<i>Corynebacterium</i>		10	5.9%
<i>E.coli</i>	6		3.6%
<i>Anterobacter agglomerans</i>	2		1.2%
<i>Actinomyces</i>	1		0.6%
Gram +ve Bacteria			
<i>Staphylococcus coagulase -ve spp</i>	18		10.7%
<i>Staphylococcus aureus</i>	6		3.6%
<i>Alpha hemolytic strep</i>	2		1.2%
<i>Enterococcus faecalis</i>	1		0.6%
Fungi			
<i>Aspergillus niger</i>		3	1.8%
Total	107	62	100%

3.3. Susceptibility Tests

Antibiotic susceptibility test was carried out for all the significant isolates which were *Proteus mirabilis*, *Pseudomonas* species, *Klebsiella pneumoniae*, and *Coagulase Staphylococcus* species. *Proteus mirabilis* showed high sensitivity rates with gentamycin (91.3%), meropenem

(89.1%), ceftazidime (87.0%), ceftriaxone (80.4%), cefotaxime (76.1%), cefoxitin (65.2%) and ciprofloxacin (67.4%) as shown in Figure 1a below. *Proteus mirabilis* showed resistance rates of 77.8% to amoxicillin-clavulanate, 68.9% to ampicillin, 91.2% to tetracycline and 78.9% to cotrimoxazole (Figure 1a).

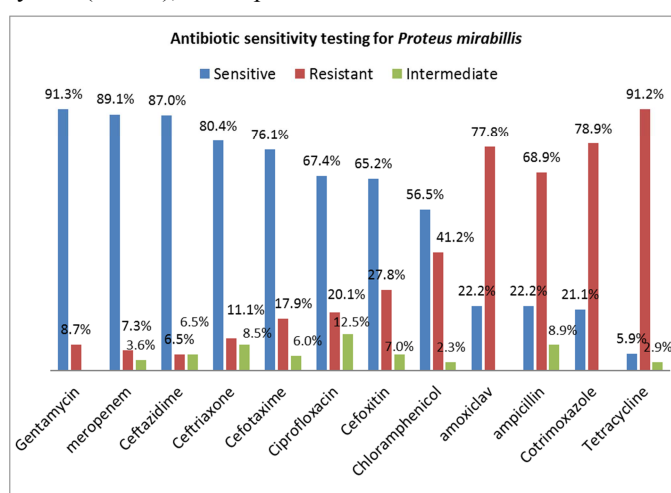


Figure 1a. Antibiotic Susceptibility testing for *Proteus mirabilis*.

Other gram-negative bacilli, *Pseudomonas* species, and *Klebsiella pneumoniae*, also showed high sensitivity rates for gentamycin (64-80%, and 100% respectively), meropenem

(66-93%, and 100% respectively), ceftazidime (>90% and 80% respectively) and ciprofloxacin (66-88%, and 84% respectively) as shown in Figure 1b, 1c and 1d below.

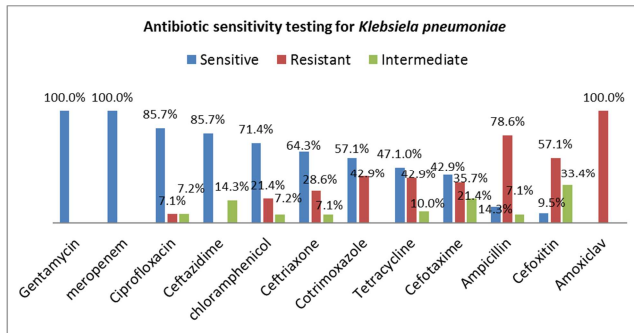


Figure 1b. Antibiotic susceptibility testing for *Klebsiella pneumoniae*.

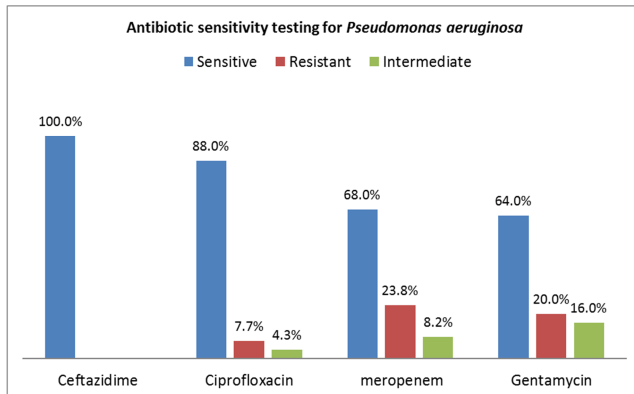


Figure 1c. *Pseudomonas aeruginosa* antibiotic susceptibility testing

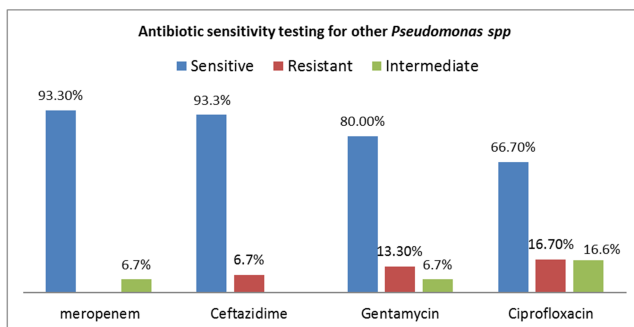


Figure 1d. Antibiotic susceptibility testing for other *Pseudomonas* species

High resistance rates were documented for gram-negative bacilli to amoxicillin-clavulanate, ampicillin, tetracycline, and cotrimoxazole (Figure 1b, 1c, 1d).

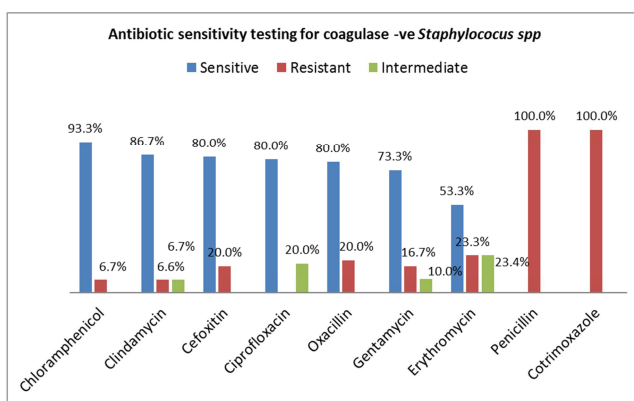


Figure 1e. Antibiotic susceptibility testing for coagulase negative *staphylococcus* species.

Gram positive cocci, coagulase negative *staphylococcus* species, showed high susceptible rates to gentamycin (73.3%), oxacillin (80%), cefoxitin (80%), chloramphenicol (93.3%), clindamycin (86.7%) and ciprofloxacin (80%) as shown in Figure 1e above. It showed the resistance of 100% to penicillin and co-trimoxazole.

4. Discussion

In this study of a 100 CSOM patients, the majority of the patients were adults that accounted for 67%. Owing to the reason that 33% of the respondents were children and that 38 adult patients (56.7% of the adults) reported onset of CSOM in childhood, it was inferred that CSOM is common in children. This is attributed to the short, wider and relatively horizontal Eustachian tube in this population.³

Children aged 0-5years accounted for 19% of the respondents (Table 1). This is similar to a study by Orji FT and Dike in 2015 where children below the age of 5 accounted for 23.8% of the patients.¹¹ It was found that CSOM was slightly more common among male patients (57%) similar to findings in a study done by Chirwa in 2014 in Malawi, where 64(61.5%) were males.¹² Other studies, however, found that CSOM was common among females than among males.¹³

The majority of the patients with CSOM in this study resided in peri-urban areas 81(81%) which are associated with a low socioeconomic status. This conforms to the notion that CSOM is a disease of those with a low socioeconomic status.^{1,14}

On examination, central perforation was the most common type of tympanic membrane perforation that was seen in 119 (84.4%) ears. This implied that most of the patients had the safe type of CSOM, tubotympanic type,¹⁵ and may explain why there was no report of complications from the patients. A very small percentage of the ears had an attic perforation (1.4%) and a marginal perforation (6.4%) which explains the small number of cholesteatoma (12%) that was found. Cholesteatoma was significantly associated with attic and marginal tympanic membrane perforation (Pearson coefficient=1).

The dominant microbiological isolate was *Proteus mirabilis* (29%), a gram-negative facultative anaerobe (Table 2). This was followed by *Pseudomonas aeruginosa* (18.9%) a gram negative aerobe. The finding of *Proteus mirabilis* as the most common isolate is similar to findings by Chirwa in 2014 in Malawi where *Proteus mirabilis* accounted for 28.6%,¹² Aduba et al in 2010 in Garissa (Kenya) where *Proteus mirabilis* accounted for 32.7%,¹⁶ and Muluye et al in 2013 in Ethiopia where *Proteus mirabilis* accounted for 27.5%.¹⁷ These findings are different from those of other studies where they found that *pseudomonas aeruginosa* was the most common isolate.^{1,5,18} The difference in the pattern of microbiological isolates may be explained by differences in the geographical conditions and population dynamics.^{8,9} *Proteus* species are widely distributed in places with poor sanitary conditions, being found in feces, decomposing meat and sewage.¹⁹ This could account for its high frequency in this study where the majority of the patients (81%) stayed in peri-urban areas which are

associated with poor sanitary conditions.

In this study, there was no significant association between age distribution and pattern of microbiological isolates. It was however found in other studies that *Proteus* spp. were the commonest isolates in pediatrics compared to adults.²⁰ There were no strict anaerobes that were isolated in this study. This finding differs from that in other studies where strict anaerobes were isolated.^{12,16} Some of the anaerobes isolated in other studies include *Bacteroides* species and *Peptostreptococcus* species.

Aspergillus niger, in only 3 (1.8%) specimens, was the only fungal microorganism that was isolated in this study. The finding of *Aspergillus niger* as an etiological agent for CSOM is supported by other studies.^{12,21,22} In other studies, *Candida* species were also isolated.^{12,21}

The sensitivity rates of *Proteus mirabilis*, gram-negative bacilli, for ciprofloxacin (a commonly used topical antibiotic), were relatively lower (67.4%) than those found in the study by Bayeh *et al* in 2011 where rates were as high as 93%.²³ However, decreased sensitivity among gram-negative bacilli to Ciprofloxacin was also noted by Jeyakumari D. *et al* in 2015.²⁴ Because Ciprofloxacin is the most commonly used otic antibiotic for CSOM, its lower sensitivity rates found in this study need to be further investigated.

As all the gram-negative bacilli, including *proteus mirabilis*, that were isolated in this study showed high susceptibility rates (>than 80%) to Ceftazidime and meropenem, these drugs can be formulated as an empirical therapy for all gram-negative bacilli in cases of complicated CSOM where an intravenous drug would be required.

Comparable to our study, high resistance rates were reported for *Proteus spp* to tetracycline (100%) and cotrimoxazole (52%) by Wariso in Nigeria.²⁵ Similarly, resistance rates of 89% for tetracycline and 64% for cotrimoxazole were reported by Bayer *et al*.²³

Gram positive cocci, coagulase negative *staphylococcus* species, showed high susceptible rates to gentamycin (73.3%), oxacillin (80%), cefoxitin (80%), chloramphenicol (93.3%), clindamycin (86.7%) and ciprofloxacin (80%). These results are comparable with those in the study by Jeyakumari, D. *et al* where they found high sensitivity rates for staphylococcus species to clindamycin (93%), Oxacillin (73%), and ciprofloxacin (73%).²⁴ Due to the high susceptibility rates, these antibiotics can be designed as empirical therapy for *Staphylococcus* species. Coagulase negative *staphylococcus spp* showed 100% resistance rates to penicillin and cotrimoxazole. Jeyakumari D *et al* in 2015 also documented high resistance rates for Staphylococcus species to penicillin (93%).²⁴

5. Conclusion

CSOM is common in both children (33%) and adults (67%). It is more prevalent in the peri-urban areas (81%) than in the urban areas (19%). *Proteus mirabilis* (29%), facultative gram-negative bacilli, was the most dominant microbiological isolate followed by *Pseudomonas aeruginosa* (18.9%), a

gram-negative aerobic bacilli. Other *Pseudomonas* species (not aeruginosa) (10.1%) and *Klebsiella pneumonia* (10.1%) were the other common gram-negative microbiological isolates. Coagulase negative *staphylococcus* species (10.7%) were the most common gram-positive microbiological isolates. The isolated microorganisms had high susceptibility rates to gentamycin (64-100%), meropenem (68-100%), ceftazidime (85-100%), ceftriaxone (64-80%), and ciprofloxacin (66-88%). High resistance rates were recorded to Amoxicillin-clavulanate (as high as 100%), ampicillin (as high as 100%), tetracycline (as high as 91.2%), cotrimoxazole (as high as 100%) and penicillin (as high as 100%). By virtue of having found a pattern of microbiological isolates in this study that is different from other studies, it can be inferred that culture and susceptibility testing for CSOM in a population/geographical area is of paramount importance for appropriate antimicrobial therapy of CSOM.

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Conflict of Interest

None of the authors have any conflicts of interest associated with the work presented in this manuscript.

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