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Ear, Nose and Throat Section

Intracranial Complications due to Chronic Suppurative Otitis Media-Atticoantral Disease with a Reference to its Microbiological Profile: A Retrospective Study

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ABSTRACT

Introduction: Intracranial Complications (ICC) in patients with Chronic Suppurative Otitis Media-Atticoantral Disease (CSOM-AAD) is a life threatening disease. Knowing the microbiological profile of the causative organisms will help in selecting empiric antibiotic.

Aim: To determine the demographic pattern and clinical presentation of patients with CSOM-AAD presenting with ICC, and to assess the microbiological profile of causative organisms.

Materials and Methods: This was a retrospective study, where medical records of all patients diagnosed with CSOM-AAD and ICC, in a tertiary teaching hospital, Manipal, Karnataka, India, between July 2012 and June 2018 were reviewed. The data regarding demographics, clinical and audiological evaluation, microbiological reports were analysed. Data was entered into Microsoft Excel sheet 2010 and was calculated as mean, median and percentages.

Results: Out of total 244 patients with CSOM-AAD, 15 presented with ICC. The age range was between 17-41 years (median age-21years). The most common complaint was headache, n=11 (73%) and the most common complication was intracranial abscess, n=10 (67%). In three cases, sampling from different sites isolated different organisms and hence a total of 20 samples (11 from ear swab, six from abscess drained pus, two from cerebrospinal fluid and one from blood culture) were analysed. Gram Negative Bacilli (GNB) were isolated most of the times, n=10 (50%) followed by Gram Positive Cocci (GPC), n=6 (30%) and anaerobes, n=2 (10%). Most of the isolated GNB and GPC were susceptible to carbapenem and vancomycin, respectively.

Conclusion: Intracranial complications are usually seen in young adults, and they present with headache, fever and ear discharge. Majority of the complications are caused by GNB. Vancomycin and carbapenem provide empiric cover for GPC and GNB, respectively.

Keywords: Cholesteatoma, Empiric antibiotic, Middle ear infection

INTRODUCTION

Chronic Otitis Media (COM) refers to the infection and inflammation of the middle ear space and mastoid that persists longer than three weeks. Cholesteatoma was first described by Duverney JG in 1683, and defined by Abramson M et al., in 1977 [1,2]. Cholesteatoma is a three dimensional epidermal and connective tissue structure, usually in the form of a sac (lined by stratified squamous epithelium and containing desquamated keratin in the center) and frequently conforming to the architecture of various spaces of the middle ear, attic and mastoid. This structure has the capacity of progressive and independent growth at the expense of the underlying bone, displacing or replacing the middle ear mucosa, and has a tendency to recur after removal.

Many theories have been proposed regarding the formation of cholesteatoma. For example, metaplasia theory described by Wendt (squamous metaplasia of respiratory epithelium lining the middle ear cleft) [3], basal cell hyperplasia theory by Lange WL (proliferation of cells in the sub epithelial layer to form micro-cholesteatoma, which ruptures the basement membrane to form cholesteatoma) [4], invasion theory by Habermann (invasion of squamous epithelium into middle ear due to marginal perforation or childhood necrotising otitis media) [5], implantation theory (traumatic implantation of canal skin to middle ear cavity) [6], retraction pocket theory by Wittmack and Tos (retractions in the pars flaccida disturbs normal growth of squamous epithelium leading to accumulation of debris, inflammation and invasion) [7]. Newly proposed theories include selective epitympanic dysventilation syndrome by Marchioni D et al., and mucosal traction theory by Jackler RK et al., [8,9].

Cholesteatoma, thus formed, harbours infectious microorganisms in 61.9%-72% of patients [10,11]. Patients usually present with persistent ear discharge with multiple unsuccessful topical treatments. Application of different topical antibiotics causes drug resistant microorganisms to emerge. Cholesteatoma, with its property to erode bone, provides an easy pathway for these micro-organisms to gain entry into the cranial cavity. This can present as meningitis, brain abscess, subdural emphysema, epidural abscess, lateral sinus thrombophlebitis and otitic hydrocephalus. Bacteriological profile of cholesteatoma has been analysed multiple times in the past [11,12], but very little evidence exists regarding the same in patients with ICC. This study aimed to determine the demographic pattern and clinical presentation of patients with CSOM-AAD presenting in ICC. Also, to assess the microbiological profile of causative organisms and determine the suitable empirical antibiotic to be used.

MATERIALS AND METHODS

The present retrospective study was conducted at the Department of Ear, Nose, Throat, Head and Neck Surgery Kasturba Medical College, Manipal Academy of Higher Education, Manipal, Karnataka, India. Data was collected between January 2018 to December 2019 from the medical records of all patients diagnosed with CSOM-AAD and ICC between July 2012 and June 2018. Approval for the study was obtained from the Institutional Ethical Committee (Protocol number-585/2017).

Inclusion criteria: All the patients that diagnosed with CSOM-AAD and ICC within the period were included in this study.

Exclusion criteria: Case records with incomplete data were excluded from the study.

As a protocol, when a patient presents to the emergency triage or outpatient clinic, a neuro-otologic assessment is performed.

- Presence of retraction pocket, frank cholesteatoma, aural polyp or auto mastoidectomy cavity on otological examination

after suctioning of the pus is necessary to diagnose COM-squamosal type.

- Presence of ICC is suspected when patients have associated headache, disorientation or any other neurological abnormality on examination.
- Radiological investigations such as contrast enhanced computed tomography or magnetic resonance imaging are sought to confirm the diagnosis.
- ICCs includes temporal lobe abscess, cerebellar abscess, sigmoid sinus thrombosis, meningitis and extradural abscess.

Initial care includes blood investigations such as complete blood count, renal function tests and blood glucose values. Ear swab for culture is taken with a sterile culture swab stick (HiCulture, HiMedia Labs, India), and empirical intravenous antibiotics covering gram positive, gram negative and anaerobic organisms are started. Neurologic or paediatric consultations are sought when found necessary. If meningitis is suspected during evaluation, lumbar puncture is performed and cerebrospinal fluid is collected and cultured. In case of intracranial abscess, intracranial drainage of abscesses is performed by the neurosurgical team, and intraoperatively drained pus is collected in a sterile container and transported to microbiology department for culture. Final diagnosis of COM- squamosal type is made intraoperatively when cholesteatoma flakes are seen in the middle ear or mastoid cavity.

Microbiological Methods

All samples are sent to the microbiology laboratory within two hours of collection for gram staining. Aerobic cultures are performed by inoculating the specimen onto 5% sheep blood agar and MacConkey agar in bacteriological incubator at 37°C for 24 hours. In the absence of any positive growth, the plates are further incubated till 48 hours. Anaerobic culture is inconsistently performed. When done, tissue specimens are inoculated in Robertson cooked meat media or thioglycolate broth and incubated in a bacteriological incubator at 37°C for 48 hours.

Culture plates with growth are separated. Isolates are further proceeded for identification and antimicrobial susceptibility testing. Identification was performed with the help of MALDI-TOF (Vitek MS) or VITEK 2 system (bioMerieux, Inc., Durham, NC). The susceptibility of these isolates to antibiotics were tested using automated micro broth dilution technique using VITEK 2 system. Antimicrobial minimum inhibitory concentrations were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2017 [13].

For each patient, data regarding demographics, symptoms at presentation, examination findings including otoscopic evaluation report were collected. Audiometric evaluations were recorded when available. Data on the bacteriological analyses of ear discharge, blood culture or culture from Cerebro-Spinal Fluid (CSF) were collected. Antibiotic sensitivity patterns were recorded and analysed.

STATISTICAL ANALYSIS

Data gathered during the study was analysed using Microsoft Excel 2010. Continuous variables were expressed in range. For age of patients, median was chosen over mean as clinical distinction in treatment plans occur before and after 18 to 20 years and a single elderly patient in the study can skew the mean value. Discrete values are expressed in bar graphs and percentage of total samples.

RESULTS

Total 244 patients were diagnosed with CSOM-AAD between 1st July 2012 and 30th June 2018, of which 15 patients had ICC. The youngest age at presentation was 17 years and the oldest was 41 years. One adolescent was identified (aged less than 18 years). More than half of the patients were aged between 18 and 29 (8 of 15 patients). Median age at presentation was 21 years. The disease had equal distribution among males and females (seven males and eight females).

Neuro-otologic assessment at presentation: Headache was the most common complaint (11 of 15). Further details of symptoms on presentation are described in [Table/Fig-1]. Active ear discharge hampered detailed otologic examination in seven of 15 patients, when the intraoperative diagnosis was made based on intraoperative evidence of cholesteatoma. Of the remaining eight, retraction pocket collecting cholesteatoma debris was the most common finding (3 of 8).

Symptom	Frequency
Headache	11
Ear discharge	9
Fever	6
Altered sensorium	3
Seizures	2
Vomiting	2
Ear pain	2
Blurring of vision	1
Neck pain	1

[Table/Fig-1]: Symptoms at presentation (N=15).

Audiometric evaluation findings: Preoperative audiogram was performed in only three of 15 cases. The hearing loss ranged between 81 and 108 decibels as measured by mean of threshold air conduction hearing at 500, 1000 and 2000 hertz pure tones.

Intracranial complications: Intracranial complication was suspected when the patient complained of headache, was disoriented or had any other neurological abnormality on examination. Radiological investigations such as contrast enhanced computed tomography or magnetic resonance imaging were sought to confirm the diagnosis. Intracranial abscess was the most common form of complication (n=10), equally distributed between temporal lobe and cerebellum (n=5 each). Other complications encountered are summarised in [Table/Fig-2].

Complication	Frequency
Intracranial abscess	10
Meningitis	3
Sigmoid sinus thrombosis	1
Extradural abscess	1

[Table/Fig-2]: Intracranial complications (N=15).

Bacteriological profile: Ear swab was the preferred method of sample collection (used in 11 of 15 patients), followed by abscess drained pus (6 of 15 patients). Other samples sent included CSF (2 of 15 patients) and blood culture (1 of 15 patients). In three cases, sampling from different sites isolated different organisms. When multiple organisms were isolated from the same person, all organisms were analysed separately for antibiotic sensitivity. All cultures were reported as monomicrobial growth in the present study.

A total of 20 samples (11 from ear swab, six from abscess drained pus, two from CSF and one from blood) were cultured (in three patients, samples taken from multiple sites isolated different organisms i.e., from ear swab, abscess drained pus, CSF and blood culture; giving a total of 20 samples for analysis). Sixteen of 20 were culture positive for aerobic bacteria, two of 20 were culture positive for anaerobic bacteria and two of 20 were culture negative after 48 hours of incubation. *Pseudomonas aeruginosa* was the most common gram negative bacilli isolated and Streptococcus species was the most common gram positive cocci isolated. There was no fungus isolated in the study. Details of isolated organisms have been given in [Table/Fig-3].

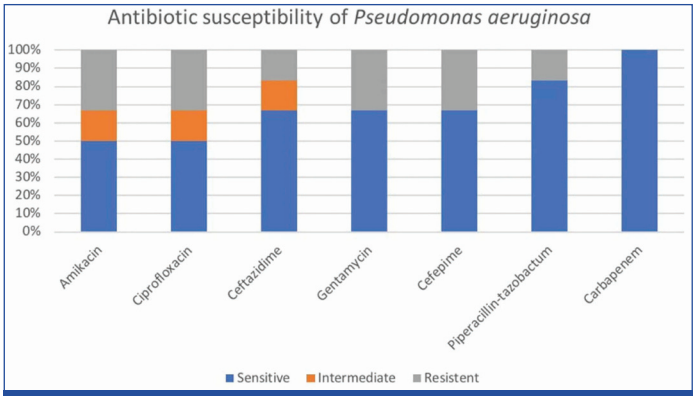
Majority of the samples from the canal grew gram negative bacteria (9 of 11). In contrast, culture from blood, CSF or pus drained from abscess showed gram negative bacteria in one sample out of nine. Majority grew gram positive cocci (4 of 9), two grew anaerobes and two showed no growth. There were three patients who underwent culture from abscess drained pus and ear swab alike. In all three, cultures from different sites grew different organisms, there was no predominance of one type of organism over other.

Sample site	Organism isolated	Total n (%)
Ear swab n=11 (55%)	**Gram negative bacteria:**	
	Pseudomonas aeruginosa	6 (30%)
	Klebsiella pneumoniae	2 (10%)
	Proteus mirabilis	1 (5%)
	Gram positive cocci:	
	Staphylococcus aureus	2 (10%)
Abscess drained pus n=6 (30%)	**Gram negative bacteria:**	
	Enterobacter cloacae	1 (5%)
	Gram positive cocci:	
	Streptococcus species	1 (5%)
	Enterococcus	1 (5%)
	Anaerobes:	
	Porphyromonas asaccharolyticus	1 (5%)
	Bacteroides fragilis	1 (5%)
	No growth	1 (5%)
Cerebrospinal fluid n=2 (10%)	**Gram positive cocci:**	
	Streptococcus species	1 (5%)
	No Growth	1 (5%)
Blood n=1 (5%)	**Gram positive cocci:**	
	Streptococcus species	1 (5%)

[Table/Fig-3]: Type and frequency of organism isolated based on site of culture (N=20).

Antibiotic sensitivity pattern:

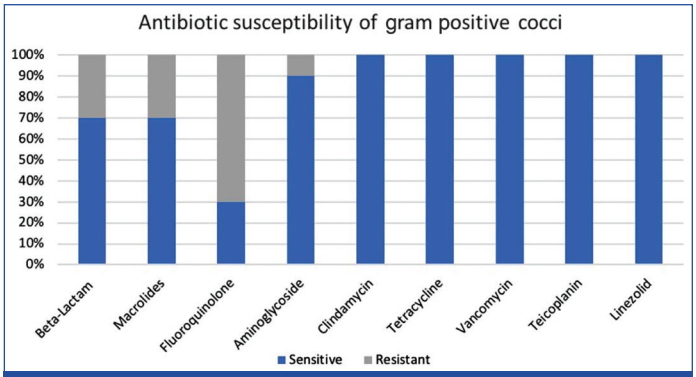
***Pseudomonas aeruginosa*:** Sensitivity to first line antibiotics was low, ranging between 50% and 66%. Eighty-three percent of isolated *Pseudomonas aeruginosa* were susceptible to carbapenem and piperacillin-tazobactum. Sensitivity pattern of isolates to antibiotics has been depicted in the [Table/Fig-4].



[Table/Fig-4]: Figure depicts the antibiotic susceptibility of Pseudomonas aeruginosa.

Enterobacteriaceae family: All isolated bacteria were sensitive to carbapenem. *Proteus mirabilis* and *Enterobacter cloacae* were sensitive to first line antibiotics, but isolates of *Klebsiella pneumoniae* were not.

Gram positive cocci: Clindamycin and tetracycline were the most effective antibiotics for gram positive cocci infection. Resistance was noted to other first line antibiotics [Table/Fig-5]. All isolated organisms remained sensitive to second line antibiotics (Vancomycin, Teicoplanin and Linezolid).



[Table/Fig-5]: Figure describes antibiotic susceptibility of gram positive cocci.

DISCUSSION

This study found that more than half the patients were aged between 18 and 29 years. This has been replicated in many other studies in the literature that has shown otogenic ICC to be more frequent in children and young adults [14-19]. Considering the long duration of time a cholesteatomatous disease takes to finally erode the tegmen, it is possible that the disease in a young adult could also have been of paediatric origin. Various factors could contribute to this hypothesis. Paediatric cholesteatoma is known to be more aggressive [20,21]. Secondly, children may find it difficult to express their symptoms as well as an adult, delaying their presentation to an otologist. Prolonged use of topical antibiotics causes more virulent strains to emerge, increasing the chance of developing ICC.

Headache, fever and ear discharge remain the most common symptoms at presentation in the present study and other studies in the literature [15,16,22-26]. This can be considered as a golden triad to look out for when treating a patient with cholesteomatous disease. Prompt treatment can then be initiated, possibly decreasing the mortality of the condition.

Abscess in brain parenchyma was the most common form of ICC in this study (10 of 15 patients), equally distributed between temporal lobe and cerebellar lobe. Intracranial abscess was identified with contrast enhanced computed tomography imaging of brain, showing a ring enhancing lesion in the temporal or cerebellar lobe with ipsilateral mastoid disease. Meningitis was the next common (three of 15 patients), identified by clinical signs of headache, nuchal rigidity and cerebrospinal fluid analysis showing neutrophils, high protein and low glucose content. Lateral sinus thrombosis was rare (one of 15 patients), diagnosed with lack of flow in a contrast enhanced computed tomography image. Extradural abscess was diagnosed in one of 15 patients, with hypodense, biconvex collection in computed tomography imaging of brain. Meningitis and abscess have been frequent form of presentation in studies from other parts of the world as well [15,16,22,24,26].

Ear swab is a quick way of procuring a culture sample in the emergency room. Abscess drained pus is a better culture sample for confirming the pathogenic agent and growing anaerobic bacteria. In this study, three patients had culture grown from different sites. In all three, different samples isolated different organisms. Therefore sampling from all sites possible is necessary for better clinical management.

As in other studies in the literature, gram negative bacteria were the most common organisms to be cultured [17,26]. Pseudomonas aeruginosa topped the list, followed by bacteria from Enterobacteriaceae family. The most common organism to cause otogenic ICC in the 21st century was *Proteus* species [17,26]. Motile gram negative bacteria are now on the rise for causing otogenic ICCs. This further explains the hypothesis that cholesteatoma debris harbor resistant microorganisms and take them to the bone eroded sterile areas as they grow.

Predominance of gram negative bacilli in the ear swab cultures and gram positive cocci in abscess drained pus culture, shows a possible polymicrobial nature of the disease.

Resistance was seen to commonly prescribed topical antibiotics like ciprofloxacin and aminoglycosides. All isolates were sensitive to carbapenem. Gram positive cocci were the second most common group of organisms to cause intracranial sepsis. Half of the gram positive cocci isolated in the present study were *Streptococcus*. Among gram positive cocci, resistance was noted for fluoroquinolone, macrolide, betalactam and aminoglycosides. All the isolated strains remained sensitive to second line antibiotics like clindamycin, tetracycline, vancomycin, teicoplanin and linezolid. Though not much literature exists about antibiotic treatment for ICC in CSOM-AAD, intravenous third generation cephalosporin with metronidazole seems to be a widely accepted choice [11]. Based on the resistance pattern seen in the present study, it is safe to start vancomycin for gram positive cocci and carbapenem for GNB empirically for any patient suspected to have otogenic ICC till culture reports become available. Also, as a primary prevention for the disease, it is reinforced to further evaluate patients with persistent ear discharge for cholesteatoma, as the disease could be eradicated prior to it reaching the cranial cavity.

Limitation(s)

Results of this study would have been more reliable if the sample size were bigger, and if all patients underwent sampling from ear, CSF or abscess drained pus and blood during early course of management. Also culture from abscess drained pus and ear swab alike could provide more information. As a retrospective study, authors could not analyse this possibility. More studies from larger centres are thus encouraged, so that clinical guidelines can be formulated and more light can be given onto the aetiopathological process of the disease.

CONCLUSION(S)

Intracranial complications due to squamosal COM is usually encountered among young adults. Headache, fever and ear discharge are common symptoms in patients with intracranial complications due to squamosal COM. Gram negative bacilli are the most commonly isolated organisms on ear swab and gram positive cocci are commonly isolated from pus in abscess. Carbapenem and vancomycin provide good empiric coverage for GNB and gram positive cocci, respectively.

REFERENCES

[1] Duverney JG. Traité de l'Organe de l'Ouie. Paris: E. Michaillet, 1683.
[2] Abramson M, Gantz BJ, Asarch RG, Litton WH. Cholesteatoma pathogenesis. Evidence for the migration theory, in, McCabe BF, Sade J, Abramson M (eds): Cholesteatoma- First International Conference. Birmingham, Alabama, Aesculapius, 1977: 176.
[3] Sadé J, Babiacki A, Pinkus G. The metaplastic and congenital origin of cholesteatoma. Acta Oto-Laryngologica. 1983;96(1-2):119-29.
[4] Lange WL. Uber beiEnstchung der Mittlohrcholesteatome. Z Hals NasOhrenheilk. 1925;11:250.
[5] Haberman J. Zur Entstehung des Cholesteatomas des Mittelhors. Arch Ohren Nasen Kehlkheilkund. 1888;27:42-50.
[6] Karmody CS, Northrop C. The pathogenesis of acquired cholesteatoma of the human middle ear. Otol Neurotol. 2012;33(1):42-47.
[7] Falk B, Magnuson B. Evacuation of the middle ear by sniffing: A cause of high negative pressure and development of middle ear disease. Otolaryngol Head Neck Surg. 1984;92(3):312-18.
[8] Marchioni D, Alicandri-Ciufelli M, Molteni G, Artioli FL, Genovese E, Presutti L. Selective epitympanic dysventilation syndrome. Laryngoscope. 2010;120(5):1028-33.
[9] Jackler RK, Santa Maria PL, Varsak YK, Nguyen A, Blevins NH. A new theory on the pathogenesis of acquired cholesteatoma: Mucosal traction. Laryngoscope. 2015;125(Suppl 4):S01-14.
[10] Likus W, Siemianowicz K, Markowski J, Wiaderkiewicz J, Kostrząb-Zdebel A, Jura-Szołtys E, et al. Bacterial infections and osteoclastogenesis regulators in men and women with cholesteatoma. Arch Immunol Ther Exp (Warsz). 2016;64(3):241-47.
[11] Mahajan T, Dass A, Gupta N, Chander J, Saini V, Pol SA. Bacteriological profile in attico-antral type of chronic suppurative otitis media. Indian J Otolaryngol Head Neck Surg. 2019;71(Suppl 2):1412-21.
[12] Madana J, Yolmo D, Kalaiarasi R, Gopalakrishnan S, Sujatha S. Microbiological profile with antibiotic sensitivity pattern of cholesteatomatous chronic suppurative otitis media among children. Int J Pediatr Otorhinolaryngol. 2011;75(9):1104-08.
[13] The Clinical & Laboratory Standards Institute (CLSI). Clinical and Laboratory Standards Institute (CLSI) guidelines. 2017. https://clsi.org/ June 03, 2019.
[14] Duarte MJ, Kozin ED, Barshak MB, Reinshagen K, Knoll RM, Abdullah KG, et al. Otogenic brain abscesses: A systematic review. Laryngoscope Investig Otolaryngol. 2018;3(3):198-208.
[15] Wu JF, Jin Z, Yang JM, Liu YH, Duan ML. Extracranial and intracranial complications of otitis media: 22-year clinical experience and analysis. Acta Otolaryngol. 2012;132(3):261-65.
[16] Dubey SP, Larawin V, Molumi CP. Intracranial spread of chronic middle ear suppuration. Am J Otolaryngol. 2010;31(2):73-77.
[17] Mustafa A, Heta A, Kastrati B, Dreshaj Sh. Complications of chronic otitis media with cholesteatoma during a 10-year period in Kosovo. Eur Arch Otorhinolaryngol. 2008;265(12):1477-82.
[18] Sennaroglu L, Sozeri B. Otogenic brain abscess: Review of 41 cases. Otolaryngol Head Neck Surg. 2000;123(6):751-55.
[19] Osma U, Cureoglu S, Hosoglu S. The complications of chronic otitis media: Report of 93 cases. J Laryngol Otol. 2000;114(2):97-100.
[20] Glasscock ME 3rd, Dickins JR, Wiet R. Cholesteatoma in children. Laryngoscope. 1981;91(10):1743-53.
[21] Palva A, Karma P, Kärjä J. Cholesteatoma in children. Arch Otolaryngol. 1977;103(2):74-77.
[22] Derić D, Arsović N, Dordević V. Pathogenesis and methods of treatment of otogenic brain abscess. Med Pregl. 1998;51(1-2):51-55.
[23] Kuczkowski J, Mikaszewski B. Intracranial complications of acute and chronic mastoiditis: Report of two cases in children. Int J Pediatr Otorhinolaryngol. 2001;60(3):227-37.
[24] Migirov L, Duvdevani S, Kronenberg J. Otogenic intracranial complications: A review of 28 cases. Acta Otolaryngol. 2005;125(8):819-22.
[25] Isaacson B, Mirabal C, Kutz JW Jr, Lee KH, Roland PS. Paediatric otogenic intracranial abscesses. Otolaryngol Head Neck Surg. 2010;142(3):434-37.
[26] Penido Nde O, Borin A, Iha LC, Suguri VM, Onishi E, Fukuda Y, et al. Intracranial complications of otitis media: 15 years of experience in 33 patients. Otolaryngol Head Neck Surg. 2005;132(1):37-42.

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