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Original Article

Definite therapy of mixed infection alleviates refractory dilemma of adult chronic suppurative otitis media

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Fungus

Abstract The characteristics, risk factors, microbial distributions and effective treatment regimens for Chronic suppurative otitis media (CSOM) patients intractable to empirical therapy were analyzed.

Adult CSOM patients of China Medical University Hospital from 2018 to 2020 were included. Subjects of refractory and non-refractory groups were investigated for characteristics of age, sex, nation, comorbidities, otomycosis, and associated complications. Risk factors, microbiology distributions, and treatment regimens were analyzed.

Twenty-six refractory patients (55.0 ± 17.7 years) and 66 non-refractory patients (54.1 ± 13.7 years) were studied. A significantly higher rate of otomycosis and CSOM complications was observed in refractory group than in non-refractory one (73.1% vs. 36.4%; $p = 0.002$; 57.7% vs. 10.6%, $p < 0.001$, respectively). Multivariate analysis revealed atopic diathesis ($p = 0.048$), otomycosis ($p = 0.003$) and CSOM complications ($p < 0.001$) were risk

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factors of refractory CSOM. Coagulase-negative staphylococci (CoNS) and methicillin-resistant *Staphylococcus aureus* (MRSA) were the prevailing pathogens. Patients of refractory group tented to have higher rates of mixed infection (42.9%% vs. 23.7%) and significantly more included fungal pathogen (19.0% vs. 2.6%; $p = 0.049$) than those of non-refractory cohort.

Topical treatment of fungus significantly improved outcome of refractory CSOM. Atopic diathesis, otomycosis, and CSOM-associated complications were risk factors of refractory CSOM. Systemic and local treatment to possible drug-resistant pathogens, likely CoNS and fungus, possible improves recalcitrant CSOM. Correspondingly, early identification of CSOM complications, routine culture and susceptibility testing and treatment of resistant bacteria and fungus are key elements to the successful management of adult CSOM.

Introduction

Chronic suppurative otitis media (CSOM) is a chronic inflammatory disease that involves the mucosa of middle ear and mastoid bone and presents with multiple episodes of persistent discharge through a perforated eardrum.[1] This presentation differentiates CSOM from other forms of chronic otitis media (COM) including chronic otitis media with effusion (COME), chronic serous otitis media and chronic secretory otitis media.[1,2] Complications of CSOM such as hearing impairment and deafness could affect up to 50% of patients, and have impacts on daily activities and learning ability of the victims.[1] CSOM was mainly prevalent in the child population.[1] A few studies on all age groups reported that the prevalence rate of CSOM reached 7.8% in India, 14% in Tanzania, and 4% in China.[1] However, the studies on adult population has been lacking.

Allergy, previous history of acute otitis media, and inhalation of passive smoke were demonstrated to be risk factors of chronic otitis media.[3] In recent decades, inappropriate antibiotic treatment, frequent attacks of upper respiratory tract infection (URTI), low socioeconomic status, and poor living quality have been common factors leading to CSOM.[1] Besides, in adults of Han implied by Wang et al., male sex, high body mass index (BMI) and smoking increased the risk to develop CSOM.[4] It is apparent that studies on adult patients with refractory CSOM are urgently needed.

The distribution of microbes isolated from otitis media (OM) changed over time and could be altered with medical intervention, such as pneumococcal vaccination.[5] Different serotypes of *Streptococcus pneumoniae* was identified in children suffered from OM.[6] The implementation of pneumococcal vaccination and stewardship of prescribed antibiotics for respiratory infections possibly contributed to the alterations of bacterial colonization, pathogen distribution, and anti-microbial resistance in subjects with OM, rhinosinusitis and tonsilitis.[7] Bacteria were the most common pathogens isolated from CSOM, and fungus was occasionally reported. Among all pathogens of CSOM, *Pseudomonas aeruginosa* and *Staphylococcus aureus* prevailed as the furthermost common pathogens in recent studies.[8]

Aural toilet, topical and systemic antibiotics have been the mainstay approach to the cure of CSOM.[1] Inadequate response to empirical therapy would lead to intractable disease.[9] The accompanied complication of cholesteatoma is frequently manifested by diffused mucosal invasion and intra-cranial invasion and needs prompt surgical interventions to overt potentially life-threatening and destructive conditions.[1,10] For patients of CSOM, persistent symptoms or signs of infection signify poorly response to treatment, and are designated as "recalcitrant" or "difficult-to-treat" CSOM.[9]

To delineate the imperative features in adult patients, we aimed to clarify the risk factors and microbiological distribution, and to accredit the effectiveness of antibiotic treatments for "refractory" CSOM of adults vs. those for non-refractory ones. The proportion of mixed pathogens was compared with that of non-refractory group. Additionally, the ultimate regimen that successfully treated refractory CSOM was annotated.

Materials and methods

Participants

This study was held at China Medical University Hospital (CMUH), a tertiary hospital in central Taiwan. An average of 85,332 outpatients and 14,928 inpatients per year during the study period was contributed by the Department of Otolaryngology, Head and neck surgery. Medical charts documented during the period from January 2018 to December 2020 were retrospectively reviewed, and those of 20 years of age or older were screened. The Committee of the Institutional Review Board of CMUH approved the data collection (CMUH110-REC1-099). The informed consent was waived due to negligible risk to personal profit of patients.

Definition and diagnosis

The subjects were diagnosed as CSOM in agreement with the definition and rule demonstrated in "World Health Organization (WHO): Chronic suppurative otitis media: burden of illness and management options".[1] Patients presented with otorrhea that has been persisted for at least two weeks were suspected of suffering from CSOM. Experienced

and qualified otolaryngologists were in charge to diagnose CSOM at outpatient clinic according to the medical history and otoscopic findings of the patients. Computed tomography (CT) was employed to investigate the possible complications damaging intra-temporal or intracranial area. In particular, cholesteatoma was diagnosed when CT showed low attenuation of soft tissue density, no contrast enhancement, and presence of bony erosion,[11] and mastoiditis was inferred when CT demonstrated fluid accumulation in mastoid cavity with or without bony erosions.[12] Surgical interventions would be conducted in case that life-threatening complications, including subperiosteal abscess or brain abscess occurred.[1]

An ear infection caused by a fungus, namely, otomycosis, was identified when a patient presented with itching, otalgia and otoscopic findings of crusted external auditory canal with mucus or pus and mass of fungal hyphae or spores.[13]

Inclusion and exclusion criteria and grouping of CSOM patients

Outpatient and inpatient medical charts of CMUH were surveyed for the diagnosis of CSOM employing the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes of 380.1—380.9, 382.0—382.9, and 384.1. A total of 10,158 patients were included and their medical charts were reviewed by two independent clinicians. Subjects with congenital structural problems such as Down syndrome or cleft palate and those lost to follow-up were excluded. Ultimately 106 adult patients who fulfilled the diagnostic criteria of CSOM and did not receive emergent operations were included for further assessment. Those patients who persistently suffered from symptoms of otorrhea, worsening of hearing and otalgia after two weeks of empirical treatment, and whose initial regimen was modified by the committed otolaryngologists were classified into the refractory groups. The non-refractory group incorporated patients who were successfully treated with empirical regimen within two weeks. A detailed flowchart is demonstrated in Fig. 1.

Demographic data recording and assessment

Two reviewers evaluated demographic data of the studied subjects via a standardized checklist which contained items of pneumococcal vaccination status, sex, age, nationality, underlying diseases, presents of otomycosis, and complications of CSOM. Of the 92 patients, 87 were from Taiwan, 1 from Indonesia, 1 from Philippines, 2 from Vietnam and 1 from Mongolia. Underlying conditions of the patients, including diabetes mellitus (DM), hypertension (HTN), hyperlipidemia, chronic kidney disease (CKD), coronary artery disease (CAD), nasopharyngeal carcinoma (NPC) status post (s/p) concurrent chemoradiation therapy (CCRT), and atopic diathesis like asthma, allergic rhinitis, and eczema, and CSOM-associated complications, were recognized by qualified specialists. All medical information was retrieved from the electronic medical charts of CMUH.

The regimen of anti-microbial agents

Antimicrobial agents were prescribed empirically for CSOM patients at the initial patient visit, and the treatment outcome was documented by attending physicians. The employed anti-microbials included topical fluoroquinolones, anti-fungal eardrops, oral penicillins, cephalosporins, macrolides, tetracyclines, and other antibiotic classes based on the principles of WHO guideline and Bailey's Head and Neck Surgery: Otolaryngology, 5th Edition. The success of CSOM treatment was defined as subjectively relief of symptoms stated by patients and objective improvement of otorrhea revealed by otoscopic findings. The CSOM patients initially received a 2-week course of empirical regimens (ER) and those who failed to respond were categorized into the refractory group. For refractory patients, physicians would change antimicrobial regimen according to clinical response or microbiology culture report as guideline suggested.[1] One regimen containing 1 to 4 drugs for both local and systemic uses was prescribed at a single visit for all patients. The regimen successfully mitigated the refractory conditions was defined as ultimate regimen (UR).

Specimen processing and pathogens identification

Routine pus collection and culture was performed when feasible for both refractory and non-refractory CSOM at outpatient visit. The in-ear pictures were photographed by Horus Digital Otoscope_DOC 100 (MiiS Inc., Hsinchu, Taiwan). Specimens were collected using a sterile ear speculum and BBL™ CultureSwab™ Plus Collection & Transport System For Aerobes & Anaerobes (BD, Franklin Lakes, New Jersey, USA) via a perforated tympanic membrane. Samples were transported to microbiological laboratory and inoculated into blood agar plate (BAP)/eosin methylene blue (EMB) agar, Columbia CAN agar (CREATIVE LIFE SCIENCE CO., LTD., New Taipei City, Taiwan) for aerobic pathogens, and anaerobic blood agar and bacteroides bile esculin/phenylethyl alcohol blood agar (CREATIVE LIFE SCIENCE CO., LTD., New Taipei City, Taiwan) for anaerobic pathogens within 24 h of specimen collection in accordance with the guideline of Clinical and Laboratory Standards Institute (CLSI).[14] Aerobes and anaerobes on culture plates were allowed to grow in 35 Celsius degree incubators for 18—24 h and 48 h, respectively. The yielded microorganism colonies from culture plates were subjected to identification employing Bruker matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) biotyper Compass (Bruker Daltonics, Billerica, Massachusetts, US). Mixed infection was diagnosed when more than one germ was isolated. Antibiotic susceptibility was determined by the BD Phoenix™ 100 Automated Microbiology System (BD, Franklin Lakes, New Jersey, USA). The resistant profiles of antibiotics were interpreted in accordance with the CLSI M100 28th ed document.[15] Mycologic culture was performed by inoculating the swab onto Mycosel Agar (CREATIVE LIFE SCIENCE CO., LTD., New Taipei City, Taiwan), Sabouraud Dextrose Agar (CREATIVE LIFE SCIENCE CO., LTD., New Taipei City, Taiwan) and Brain Heart Infusion Agar (BHI Agar) with Blood and Antibiotic (CREATIVE LIFE SCIENCE

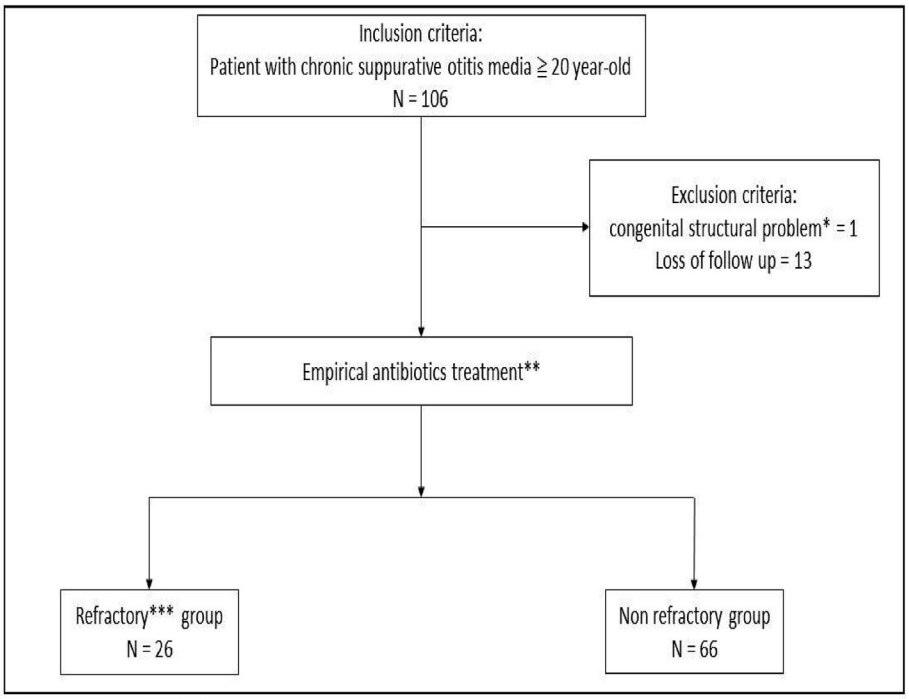

Figure 1. Inclusion, exclusion criteria and grouping.
*Down syndrome, cleft palate.
**Use alone or combination use: ofloxacin otic solution, oral cephalosporins, penicillins, macrolides, tetracyclines, clindamycin, sulfamethoxazole + Trimethoprim, fusidic sodium, rifampicin, linezolid.
***no improvement after 2 weeks of treatment on initial regimens, not related to side effect nor changed according to culture data.

CO., LTD., New Taipei City, Taiwan). The plates were stored in incubators without carbon dioxide (CO_2) for the Mycosel Agar and Sabouraud Dextrose Agar at 25 °C and for Brain Heart Infusion Agar (BHI Agar) with Blood and Antibiotic at 35 °C. Colonies from culture plates were subjected to the preparation for identification using slide-culture method. Lactophenol cotton blue staining was applied for morphology examination and identification of fungus. CSOM and otomycosis pictures are demonstrated in Fig. 2.

Statistical analysis

We compared variables between refractory and non-refractory groups, including age, gender, nationality, co-morbidity, otomycosis and CSOM associated complications. Continuous variables were presented by means and standard deviation (SD), and categorical variables were shown as n (%). Independent *t* test was performed to compare means between two groups. Fisher's exact test was applied

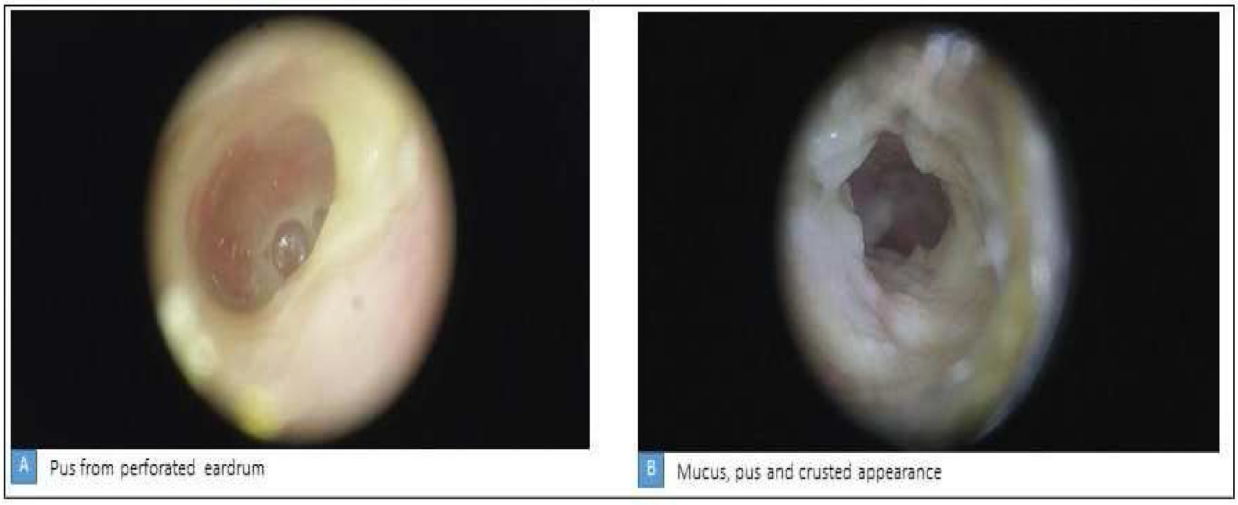

Figure 2. Typical images recorded by Horus Digital Otoscope_DOC 100. (A) Pus from perforated eardrum. (B) Mucus, pus and crusted appearance.

to analyze dichotomous variables. Binomial logistic regression for univariate and multivariate analysis was used to evaluate risk factors associated with refractory conditions. Odds ratio (OR) and 95% confidence interval (CI) were listed, and a *P* value less than 0.05 was defined as statistically significant. Statistical analysis employed SPSS statistics version 22 software (IBM® SPSS® Statistics, Illinois, Chicago, US).

Results

Demographic data

A total of 106 patients diagnosed as CSOM were enrolled in this study. Fourteen patients were excluded due to cleft palate (1 patient) or loss to follow-up (13 patients). Twenty-six patients were classified in refractory group (N = 26) and 66 patients in non-refractory group (N = 66), as shown in Fig. 1. None of the included patients had a history of pneumococcal vaccination. The mean ages in the refractory and non-refractory groups were 55.0 ± 17.7 years (mean ± SD; ranging from 22 to 89 years) and 54.1 ± 13.7 years (mean ± SD; ranging from 23 to 81 years), respectively. The ratio of male population was 38.4% (10/26) in refractory group and 34.8% (23/66) in the non-refractory group. Most of the included patients were Taiwanese in both refractory (24/26; 92.3%) and non-refractory (63/66; 95.5%) groups. HTN, previous OM infection, and DM were the most common comorbidities for both groups of patients, however, no significant difference was observed between groups. The prevalence rate of otomycosis in refractory group (73.1%; 19/26) was 2.0 times higher than that in non-refractory group (36.4%; 24/66, $p = 0.002$). Fifteen of 26 patients (57.7%) of refractory group evolved complications of CSOM, which was a 5.4-fold increase from that of non-refractory group (7/66, 10.6%, $p < 0.001$). Data are summarized in Table 1.

Microbiology analysis between groups

A total of 87 isolates were obtained from 59 patients available for culture studies, of whom 37 subjects (62.7%) had mono-pathogen, 18 (30.5%) had mixed pathogens and 4 had no micro-organism growth. For patients with mixed pathogens, two germs were isolated from 6 patients; three from 10 subjects and four from 2 patients. As shown in Table 2A, the ratio of patients with mixed pathogens in refractory group (9/21, 42.9%) was 1.8 times higher than that in non-refractory group (9/38, 23.7%). Similarly, the ratio of fungal pathogen was 7.3 times higher in refractory group than in non-refractory one (4/21, 19.0% vs. 1/38, 2.6%; $p = 0.049$; Table 2A).

Out of 39 isolates from patients in the refractory group, 26 (66.7%) isolates were stained Gram-positive, 5 (12.8%) Gram-negative and 8 (20.5%) other pathogens. Among all pathogens, coagulase-negative staphylococci (CoNS) were most frequently isolated (n = 16), following *Staphylococcus epidermidis* (n = 5), Methicillin-resistant *S. aureus* (MRSA, n = 4), *P. aeruginosa* (n = 3) and *Aspergillus niger* (n = 3) were other frequent pathogens. Similar distributions were observed in non-refractory group, 37 isolates

Table 1 Characteristics of CSOM patients in refractory and non-refractory groups.

	Refractory group (n = 26)	Non-refractory group (n = 66)	*P* value
Age, mean (SD)	55.0 (17.7)[a]	54.1 (13.7)[a]	0.800
Male, n (%)	10 (38.4)	23 (34.8)	0.811
Nation, n (%)			
Taiwan	24 (92.3)	63 (95.5)	0.619
Indonesia	0 (0)	1 (1.5)	1.000
Philippines	1 (3.8)	0 (0)	0.283
Vietnam	1 (3.8)	1 (1.5)	0.488
Mongolia	0 (0)	1 (1.5)	1.000
Comorbidities, n (%)			
Diabetes mellitus	5 (19.2)	7 (10.6)	0.309
Hypertension	6 (23.1)	14 (21.2)	1.000
Hyperlipidemia	1 (3.8)	2 (3.0)	1.000
Chronic kidney disease	2 (7.7)	1 (1.5)	0.192
Coronary artery disease	1 (3.8)	1 (1.5)	0.488
NPC s/p CCRT	2 (7.7)	6 (9.1)	1.000
Previous otitis media Infection, n (%)	6 (23.1)	13 (19.7)	0.777
Atopic diathesis (asthma, allergic rhinitis, eczema)	3 (11.5)	1 (1.5)	0.067
Otomycosis, n (%)	19 (73.1)	24 (36.4)	0.002
Associated complications, n (%)	15 (57.7)	7 (10.6)	<0.001
Mastoiditis	8 (30.8)	6 (9.1)	0.020
Cholesteatoma	9 (34.6)	3 (4.5)	<0.001
Cranial nerve involvement	1 (3.8)	0 (0)	0.283

[a] Age was normally distributed and was presented as mean (SD).
CSOM: chronic suppurative otitis media; SD: standard deviation; NPC: nasopharyngeal carcinoma; s/p CCRT: status post concurrent chemoradiotherapy.

Table 2A Applicable CSOM patients with mono- and mixed pathogens yielded from microbiologic cultures of refractory vs. non-refractory groups

	Refractory group (n = 21)	Non-refractory group (n = 38)	*p* value
Mono-pathogen, n (%)	12 (57.1)	25 (65.8)	0.580
Mixed pathogens, n (%)	9 (42.9)	9 (23.7)	0.149
Mixed bacterial infection, n (%)	5 (23.8)	8 (21.1)	1.000
Fungus isolated from mixed infection, n (%)	4 (19.0)	1 (2.6)	0.049

(77.1%) were Gram-positive, 7 (14.6%) Gram-negative and 4 (8.3%) other pathogens; the prevailing pathogens were CoNS (n = 13), MRSA (n = 7), Methicillin-sensitive *S. aureus* (MSSA, n = 6), *S. epidermidis* (n = 4), *Corynebacterium striatum* (n = 2) and *P. aeruginosa* (n = 2). The distributions of pathogens were not significantly different between groups and are shown in Table 2B.

ER and successful UR for patients of the refractory group

All of the patients of the refractory group received their ER as initial treatment, and a revised regimen, UR, was administered to those who responded unfavorably to ER. Complicated patients who received emergent surgical intervention were excluded from this study, and every patient of the refractory group received UR prescription. The proportion of UR/ER in refractory group was 92.9%. A total count of 107 anti-microbial agents were applied to 26 refractory patients to treat CSOM in either ER or UR formula, attributed to 26 regimens. Each regimen contained up to 3 drugs; 1-, 2-, and 3-drug regimens, respectively, were prescribed to 5, 20, and 1 patient as ER, and to 4, 11, and 11 patients as UR.

Tarivid® otic solution (ofloxacin 3 mg per ml; Daiichi-Sankyo, Nihonbashi, Tokyo) was applied to CSOM patients half the ratio in UR group than in ER one (12/59, 20.3% vs. 20/48, 41.7%; $p = 0.020$). In contrast, MYCOMB® otic solution, containing triamcinolone acetonide 1.0 mg, neomycin Sulfate 2.5 mg, gramicidin 0.25 mg and nystatin 100,000 International unit in each 5 ml bottle (Sinphar Group, Yilan, Taiwan), was prescribed 6.3 times more in UR than in ER (16/59, 27.1% vs. 2/48, 4.3%; $p = 0.002$). For systemically administered antibiotics, amoxicillin/clavulanic acid (n = 9), clindamycin (n = 5) and sulfamethoxazole/trimethoprim (n = 4) were frequently prescribed in ER, and similarly, amoxicillin/clavulanic acid (n = 8) and sulfamethoxazole/trimethoprim (n = 4) in UR. There was no statistical difference between groups on the individual drug of systemic anti-bacterial medications ($p = 0.440$). Targeted therapies for culture-proved tuberculosis and fungal infections were applied for 1 and 3 subjects, respectively, and were not different between UR and ER ($p = 1.000$ and 0.251, respectively). The details of regimen in each group are summarized in Table 3.

Risk factors analysis

Univariate analysis revealed that there was no significant difference between refractory and non-refractory groups on the distributions of age (OR: 0.996, CI: 0.966—1.027, $p = 0.797$), sex (OR: 0.856, CI: 0.335—2.187, $p = 0.745$), DM (OR: 2.007, CI: 0.574—7.012, $p = 0.275$), HTN (OR: 1.114, CI: 0.376—3.303, $p = 0.845$), hyperlipidemia (OR: 1.280, CI: 0.111—14.753, $p = 0.843$), chronic kidney disease (OR: 5.417, CI: 0.469—62.500, $p = 0.176$), coronary artery disease (OR: 2.600, CI: 0.157—43.183, $p = 0.505$), NPC s/p CCRT (OR: 0.833, CI: 0.157—4.422, $p = 0.830$), previous OM infection (OR: 1.223, CI: 0.409—3.658, $p = 0.719$) and atopic diathesis (OR: 0.118, CI: 0.012—1.191, $p = 0.070$). Applying multivariate analysis to

Table 2B Isolates of microbiologic cultures from applicable CSOM patients of refractory vs. non-refractory groups

	Refractory group (n = 39)*	Non-refractory group (n = 48)*	*p* value
Gram-positive bacteria, n (%)	26 (66.7)	37 (77.1)	0.338
Methicillin-sensitive Staphylococcus aureus (MSSA)	1 (2.6)	6 (12.5)	0.124
Methicillin-resistant Staphylococcus aureus (MRSA)	4 (10.3)	7 (14.6)	0.748
Staphylococcus epidermidis	5 (12.8)	4 (8.3)	0.507
Staphylococcus lugdunensis	0 (0)	1 (2.1)	1.000
Other Coagulase-negative staphylococci (CoNS)	11 (28.2)	13 (27.1)	1.000
Enterococcus faecalis (non-vancomycin resistant)	1 (2.6)	0 (0)	0.448
Corynebacterium striatum	0 (0)	2 (4.2)	0.500
Corynebacterium jeikeium	0 (0)	1 (2.1)	1.000
Corynebacterium amycolatum	1 (2.6)	0 (0)	0.448
Corynebacterium tuberculostearicum	1 (2.6)	1 (2.1)	1.000
Corynebacterium falsenii	1 (2.6)	0 (0)	0.448
Propionibacterium avidum	0 (0)	1 (2.1)	1.000
Unspecified Gram-positive bacillus (GPB)	1 (2.6)	1 (2.1)	1.000
Gram-negative bacteria, n (%)	5 (12.8)	7 (14.6)	1.000
Pseudomonas aeruginosa	3 (7.7)	2 (4.2)	0.653
Escherichia coli	0 (0)	1 (2.1)	1.000
Serratia marcescens	0 (0)	1 (2.1)	1.000
Enterobacter aerogenes	1 (2.6)	0 (0)	0.448
Burkholderia cepacia	1 (2.6)	0 (0)	0.448
Achromobacter ruhlandii	0 (0)	1 (2.1)	1.000
Acinetobacter baumannii	0 (0)	1 (2.1)	1.000
Acinetobacter ursingii	0 (0)	1 (2.1)	1.000
Others, n (%)	8 (20.5)	4 (8.3)	0.125
Mycobacterium tuberculosis complex	1 (2.6)	0 (0)	0.448
Aspergillus niger	3 (7.7)	1 (2.1)	0.321
Aspergillus flavus	1 (2.6)	1 (2.1)	1.000

Table 2B (*continued*)

	Refractory group (n = 39)*	Non-refractory group (n = 48)*	*p* value
Candida parapsilosis	1 (2.6)	1 (2.1)	1.000
Candida metapsilosis	1 (2.6)	1 (2.1)	1.000
Fusarium species	1 (2.6)	0 (0)	0.448

*A total of 39 and 48 isolates identified from mono- and mixed pathogen cultures of refractory and non-refractory groups, respectively, were analyzed.

Table 3 Anti-microbials employed for the treatment of patients in refractory group.

	Drugs of empirical regimen (n = 48)	Drugs of ultimate regimen (n = 59)	*p* value
Topical agents, n (%)			
Ofloxacin*	20 (41.7)	12 (20.3)	0.020
Triamcinolone/Neomycin/ Gramicidin/nystatin*	2 (4.3)	16 (27.1)	0.002
Systemic anti-microbials, n (%)			
Anti-bacterial	26 (54.2)	27 (45.8)	0.440
Ciprofloxacin**	1 (2.1)	2 (3.4)	
Moxifloxacin**	0 (0)	1 (1.7)	
Amoxicillin**	2 (4.2)	1 (1.7)	
Dicloxacillin**	0 (0)	1 (1.7)	
Amoxicillin/clavulanic acid**	9 (18.8)	8 (13.6)	
Piperacillin Sodium + Tazobactam Sodium***	0 (0)	1 (1.7)	
Cephradine**	1 (2.1)	0 (0)	
Ceftibuten**	1 (2.1)	0 (0)	
Erythromycin**	1 (2.1)	0 (0)	
Doxycyclin**	0 (0)	2 (3.4)	
Clindamycin**	5 (10.4)	2 (3.4)	
Sulfamethoxazole/ Trimethoprim**	4 (8.3)	4 (6.8)	
Fusidic sodium**	1 (2.1)	1 (1.7)	
Rifampicin**	1 (2.1)	2 (3.4)	
Linezolid**	0 (0)	1 (1.7)	
Teicoplanin***	0 (0)	1 (1.7)	
Anti-tuberculosis, n (%)	0 (0)	1 (1.7)	1.000
Rifampin/isoniazid / pyrazinamide/ ethambutol**	0 (0)	1 (1.7)	
Anti-fungal, n (%)	0 (0)	3 (5.1)	0.251
Fluconazole**	0 (0)	2 (3.4)	
Itraconazole**	0 (0)	1 (1.7)	

A total of 26 refractory patients was included, empirical regimen (ER) and ultimate regimen (UR) contributed 48 and 59 prescriptions, respectively.
*otic solution.
**oral medicine.
***intravenous injection.

evaluate factors for patients refractory to ER treatment, atopic diathesis had a markedly increased risk (OR: 24.483, CI: 1.026–584.153, $p = 0.048$). Moreover, otomycosis (OR: 4.750, CI: 1.745–12.928, $p = 0.002$ in univariate analysis; OR: 8.260, CI: 2.022–33.744, $p = 0.003$ in multivariate analysis) and CSOM-associated complications (OR: 11.494, CI: 3.810–34.673, $p < 0.001$ in univariate analysis; OR: 26.912, CI: 5.521–131.176, $p < 0.001$ in multivariate analysis) were more prevalent in refractory group than in non-refractory group. Detailed data are summarized in Table 4.

Discussion

This is the first study to elucidate the refractory subgroup of CSOM in adult patients by analyzing its characteristics and risk factors, causative pathogens, and the effective treatment regimen. The presence of fungal infection in the auditory canal, the complications associated with CSOM, and a history of atopic diathesis were independent risk factors related to unresolved condition of refractoriness. The ratio of infections by mixed pathogens with included fungi was significantly higher in CSOM patients of refractory group than in those of non-refractory one. It is plausible that fungus play a vital role seeing that the addition of topical anti-fungal agents to cope with refractory CSOM in this study reached a satisfactory outcome.

Otomycosis was present in most CSOM patients who were refractory to empirical treatment in this study. Fungal otitis externa at times invades the middle ear cavity via perforated eardrum and presents with ear discharge and fullness,[16] which resembles the presentation of bacterial CSOM. Fungi entered the middle ear space and colonized in cholesteatoma, from which recognizable development of invasive disease could unveil.[17] Another large-scale study from India, data collected during the period of 2006–2017, demonstrated that approximately 0.20% (15/7426 subjects) with bacterial CSOM were eventually diagnosed with fungal infection, and in 13.3% of which *Aspergillus* spp., *Candida* spp., or *Mucorales* spp. could be isolated.[18] In consequence, superimposed fungal infection should be highly suspected if CSOM was refractory to local or systemic antibacterial therapy. Similarly, Mittal, R. et al. demonstrated the microbial distributions of CSOM to be mixed bacteria and fungus in nature.[8] Fungus was present in 44.4% of mixed infections and was the second prevalent isolate in patients with refractory CSOM in our study (Tables 2A and 2B). Moreover, CSOM intractable to antibiotic treatment could be alleviated by the application of local or systemic anti-fungal agents, for which were included in up to 32.2% of UR and achieved a good response. It is likely that the inadequate ER response in the refractory cohort could be attributed to the insufficient antifungal coverage in ER. Topical antibiotics and humid atmosphere are presumable factors associated with the progress of otomycosis.[19] CSOM patients refractory to treatment had a high prevalence rate of otomycosis in this study, and in contrast, those non-refractory had only 2 subjects suffering from mixed bacterial and aspergillus infection. All of the included patients with otomycosis were treated successfully with regimens containing anti-bacterial and anti-fungal drugs. It is of

Table 4 Binomial logistic regression for the analysis of associated risk factors.

	Univariate analysis			Multivariate analysis		
Variables	OR	95% CI	p value	Adjusted OR	95% CI	p value
Age	0.996	0.966–1.027	0.797	1.008	0.959–1.060	0.747
Sex	0.856	0.335–2.187	0.745	1.251	0.333–4.698	0.740
Diabetes mellitus	2.007	0.574–7.012	0.275	3.134	0.425–23.092	0.262
Hypertension	1.114	0.376–3.303	0.845	0.489	0.080–2.992	0.439
Hyperlipidemia	1.280	0.111–14.753	0.843	3.894	0.073–208.943	0.503
Chronic kidney disease	5.417	0.469–62.500	0.176	1.101	0.047–26.047	0.952
Coronary artery disease	2.600	0.157–43.183	0.505	0.893	0.034–23.476	0.946
NPC s/p CCRT	0.833	0.157–4.422	0.830	2.128	0.222–20.439	0.513
Previous otitis media infection	1.223	0.409–3.658	0.719	3.356	0.691–16.302	0.133
Atopic diathesis (asthma, allergic rhinitis, eczema)	0.118	0.012–1.191	0.070	24.483	1.026–584.153	0.048
Otomycosis	4.750	1.745–12.928	0.002	8.260	2.022–33.744	0.003
Associated complications	11.494	3.810–34.673	<0.001	26.912	5.521–131.176	<0.001

NPC: nasopharyngeal carcinoma; s/p CCRT: status post concurrent chemoradiotherapy; OR: Odds ratio; CI: confidence interval.

concern that CSOM patients should be investigated for fungus as a possible pathogen.

S. aureus and *P. aeruginosa* have been considered as typical pathogens of CSOM.[20] In the study conducted by Heilmann C et al., CoNS was shown to be virulent and participated in the process of human infection. It could invade the middle ear cavity and cause CSOM, especially in the victim with broken mucosa barrier.[21] It was in parallel with our study that CoNS was demonstrated as the most prevailing microbe.[22] Employing proper aseptic procedures to sample and harvest middle ear pus through perforated eardrum in our investigation, CoNS represented 41% and 37.5%, respectively, of isolates from refractory and non-refractory patients of CSOM. Similarly, Mofatteh et al. reported the prevalence rate of CoNS as 35.7% in CSOM.[20] With a growing evidence of medium-to-low pathogenicity, it is reasonable that, rather than a usual habitant, CoNS likely invaded the ear tissue and caused CSOM. Moreover, standard treatment of CSOM, including topical ofloxacin uses, could suppress the colonization and infection of common pathogens of CSOM and lead to the emergence of opportunistic pathogens. As demonstrated in Table 3, ofloxacin, amoxicillin/clavulanic acid, clindamycin, and sulfamethoxazole/trimethoprim composed 79.2% of ER, which mostly covered pathogens including MSSA and *P. aeruginosa*, and partially MRSA.[23] This practice possibly led to the emergence of novel resistant pathogens, especially CoNS. Among few studies reporting the susceptibility of CoNS of CSOM, Mofatteh et al. reported a rate of CoNS resistance to oxacillin reaching 91.7%.[20] Hence, methicillin resistance should be justified for the appropriateness of empirical and target therapy of CSOM patients. Since repeated regimens without diversification for recurrent episode of infection could lead to resistant microbes,[24] routine culture and sensitivity testing are needed to guide the treatment of prolonged CSOM infection.

Although one study from China showed the effectiveness of systemic anti-fungal agents to otomycosis,[25] MYCOMB® otic solution was applied to patients with fungal and mixed ear infections and achieved a favorable outcome in this study. Eardrops into middle ear cavity have local effects on the successful treatment of CSOM, yet they could cause ototoxicity. Likewise, Burow's solution, an over-the-counter drug containing 13% of aluminum acetate has both anti-bacterial and anti-mycotic properties and is used to cope with local infection of otitis externa and COM.[26] Two patients of otitis media with tympanic membrane perforation were reported to be presented with ototoxicity after applying Burow's solution.[27] MYCOMB® otic solution was employed in this study due to regional availability and few reported ototoxicity. Guzman et al. disclosed that triamcinolone acetonide did not cause toxic effect. On the contrary, it provided protection against ototoxic agents in an animal model.[28] Only one case was unveiled to suffer from sensorineural hearing loss after applying a combination of triamcinolone, neomycin, gramicidin and nystatin cream locally.[29] Thus, current evidence of possible ototoxicity of MYCOMB® otic solution was insufficient.

Mastoiditis, cholesteatoma, and cranial nerve involvement are severe complications of CSOM and cause morbidity or mortality.[10] A considerable number of patients in our refractory cohort suffered from these troublesome conditions, and have prolonged and difficult-to-treat disease. Notably, cholesteatoma possessed the ability to migrate and invade the normal ear tissues, and Singh GB et al. demonstrated that 42.5% of studied cholesteatoma cases had fungal component that could cause lethal diseases.[17] Biofilm formation and fungal invasion likely occurred in CSOM patients with cholesteatoma and resulted in recalcitrant and recurrent conditions,[30,31] and local application of antibacterials and anti-fungals with good penetration into biofilms could be of help.

This study disclosed that atopic diathesis was associated with recalcitrant response to initial empiric anti-microbial therapy in adults with CSOM; MacIntyre et al. acknowledged atopic diseases, including eczema and asthma are risk factors of otitis media in the early life of childhood.[32] Although Wang et al. argued that characteristics of Han adults, such as sex and high BMI, were related to the chronic deterioration of otitis media,[4] however, we did not recognized a difference in age, sex, nation, or comorbidity between refractory and non-refractory groups. Since the refractory features of studied CSOM subjects defined in this study were not established by Wang et al. or in other

literature, further researches are needed to clarify the unsolved problems.

There are several limitations for this study. First of all, pus culture was performed based on clinical needs but not at routine visits prior to antimicrobial treatment. Correspondingly, the variations of microbial species in CSOM between the initial and post-treatment visits were not evaluated to correlate with clinical outcomes. Secondly, pus was sent to central lab only for culture of aerobic pathogens and without immediate smear for Gram stain to recognize phagocytosis of pathogens. This may not reflect the real distribution of all pathogens for targeted antimicrobial therapy. Thirdly, the specimens were subjected to mycologic culture and identification but not treated routinely with potassium hydroxide (KOH) for direct examination. Lastly, testing data for antimicrobial resistance of clinical isolates was executed for Gram-negative bacilli (GNB) and *S. aureus*, but not for CoNS. Seeing that identification and resistant patterns of all isolates were not fully defined, further research employing prospective design on refractory CSOM is needed to solve the intractable problems of CSOM.

Conclusion

In conclusion, the main pathogens responsible for CSOM were *Staphylococcus* species, especially CoNS, followed by *P. aeruginosa* in both refractory and non-refractory groups. Mixed pathogens with included fungi were more prevalent in patients of refractory group than in those of non-refractory group. It will be of value to implement routine culture to identify bacterial resistance and fungal pathogen when CSOM patients are intractable to empirical treatment. Simultaneously, atopic diathesis, otomycosis and CSOM-associated complications were risk factors of CSOM patients to develop refractory condition. In that event, the recognition of complications, pathogens, and resistance profiles in CSOM victims and early interventions including targeted antimicrobial therapy will possibly reverse the refractory status. Further studies are warranted to solve this challenging issue.

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