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# Microbiology of Chronic Otitis Media

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author OJA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors OJA, NOE, OKF managed the analyses of the study and literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

Middle ear swabbed samples from patients (aged ≤1 yr to ≥ 60 yrs) attending Ear, Nose and Throat (ENT) clinics at Uyo and Ikot Ekpene with chronic otitis media (COM) were analyzed microbiologically. The occurrences of DNase, β-lactamase, haemolysin production and susceptibility of the isolates obtained to antibiotics were determined using standard techniques. *Staphylococcus aureus* 65 (24.7%), *Pseudomonas aeruginosa* 53 (20.1%). *Proteus mirabilis* 22 (8.4%), *Streptococcus pneumoniae* 21 (7.9%), coagulase negative *Staphylococcus* spp 19 (7.2%), *Klebsiella pneumoniae* 18(6.8%), *Escherichia coli* 17 (6.5%), *Proteus vulgaris* 10 (3.8%), *Serratia marcescens* 9 (3.4%), *Streptococcus pyogenes* 10 (3.8%), *Enterobacter* spp 8 (3.0%), *Morgenella morganii* 7 (2.7%), and *Bacillus substilis* 4 (1.5%) were obtained. Fungal isolated were 29.2% *Aspergillus niger,* 6.3% *Aspergillus flavus,* 22.9% were *Candida albicans,* 10.4% *Candida* spp, 17.7% *Cryptococcus neoformans* and 13.5% *Fusarium* spp. *S. aureus* and *B. substilis* had the highest and lowest frequency of occurrences in both sexes, respectively. Of 31 isolates that showed positivity for β haemolysis, 7 (70.0%) were *S. pyogenes* and 15 (23.1%) from *S. aureus*. *S. aureus* was the highest DNase producers, followed by *S. pyogenes* 4 (40.0%) and CoN *Staphylococcus*

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spp. 1 (5.3%). β-lactamase detection ranged from 28.6% in *M. morganii* to 55.6% in *S. marcescens*. Haemolysin and DNase producing *C. albicans* and other *Candida* spp were isolated. Between 55.9% and 60.8% isolates were sensitive to ceftriaxone, cefotaxime and ceftazidime, while between 44.4% to 62.5% of *Enterobacter* spp and *S marcescens* were resistant to penicillin and ceftazidime. Of the 243 MDR bacteria, 136 (56.0%) were resistant to 4-8 antibiotics with indices ranging from 0.17 to 0.67. *C. albicans* and *A. niger* were more sensitive to nystatin than other fungal spp, while between 30.2% to 39.6% of the fungi were resistant to fluconazole and ketoconazole. Conclusively, this study showed the need to find compounds that potentiate antimicrobial activity against multidrug resistant organisms especially those associated with chronic otitis media.

*Keywords: Chronic; otitis media; betalactamase; antibiotics; susceptibility; haemolysis; bacteria.*

## 1. INTRODUCTION

Otitis media is the infection of the middle ear due to pathogenic micro-organisms [1]. Otitis media can be either acute or chronic, of which each is subdivided and classified as suppurative or non-suppurative, depending on the presence of fluid and also based on the complication [2]. The etiopathogenesis of chronic otitis media (COM) is multifactorial, including infection, anatomical, immunological, genetical and environmental factors [3,4]. Sources of infection are dependent on the route by which infection reaches the middle ear [5]. Children are much more susceptible to otitis media since the eustachian tube is shorter and at more of a horizontal angle than in the adults [6]. The bacteria associated with otitis media include *P. aeruginosa, E. coli, S. aureus, S. pyogenes,* and *Klebsiella* spp [7,8]. These bacteria are infrequently found in the skin of the external canal but may proliferate in the presence of trauma or inflammation [9]. It has been reported that *A. niger, C. albicans* and *Aspergillus* spp caused COM [10]. The patients with COM present the classic "earache", severe and continuous pain, fever, loss of balance, draining of fluid in the ear, brain abscess and even death if a severe infection goes untreated long enough [1,11]. COM has been found to be the single major cause for conductive deafness and also responsible for 1.5% of speech disorders [12]. Recent developments in the treatment of patients with otitis media include the use of cephalosporins and newer topical flouroquinolones [10]. Flouroquinolone antibiotics target DNA gyrase and topoisomerase IV by disrupting DNA synthesis and causing lethal double-strand DNA breaks during DNA replication [13]. Treatment of fungal infections has been less successful because eukaryotic fungal cells are much more similar to human cells than are in bacteria [14]. Despite the relatively low therapeutic index, a few antifungal drugs such as ketoconazole, fluconazole and nystatin are useful in treating many fungal diseases [15,16].

Microorganisms' mechanisms of overcoming the activities of antimicrobial agents include the alteration of penicillin-binding proteins, alteration of DNA gyrase targets, permeability mutations, active efflux and ribosomal modification [17]. *C. albicans* mutations in the ergosterol biosynthetic pathway and resistance to several antifungal agents have been reported [18,19]. Multidrug-resistant bacteria (MDR) are important concern to the clinicians, as it is the major cause of failure in the treatment of infectious diseases, increased morbidity, and mortality and the evolution of new pathogens [20]. The most common causes of bacterial resistance to β-lactam antibiotics are the production of β-lactamases, the presence of plasmid and mutation [21].

Consequently, this study aimed at isolating micro-organisms associated with chronic otitis media infections, determining their virulence factors and also susceptibility to chemotherapeutic agents.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Samples

One hundred and seventy-six (176) middle-ear samples from patients (aged ≤1 year to ≥ 60 years) attending Ear, Nose and Throat (ENT) clinics at Uyo and Ikot Ekpene, who had not received antibiotic therapy for the previous three days and were certified by ENT specialists to have chronic otitis media (COM) were collected aseptically using sterile swab sticks from January to December, 2011 The samples were transported in nutrient broth to the Microbiology Laboratory for microbiological analyses.

## 2.2 Bacteriology of the Samples

The middle ear swabbed samples inoculated into nutrient broth for 4-6 hr were later streaked onto plates of Nutrient Agar, Blood Agar, MacConkey Agar, Chocolate Agar, Eosin Methylene Blue Agar, and Mannitol Salt Agar and incubated aerobically at 37℃ for 24 hr. After incubation, the colonies on the positive plates were subcultured onto Petri dishes containing nutrient agar and incubated at 37℃ for 24 hr. Pure culture of isolates were streaked onto nutrient agar slants, incubated at 37℃ for 24 hr and later stored in the refrigerator at 4℃ for characterization and identification. All isolates were Gram stained and subjected to biochemical tests using standard methods [22].

## 2.3 Mycology of the Samples

The middle ear swabbed samples inoculated into nutrient broth for 4-6 hr were also streaked onto plates of Sabourand Dextrose Agar (SDA) supplemented with 2 drops of streptomycin (5 µg/ml). The plates were incubated aerobically at 25℃ for 3-7 days. After incubation, cultures were examined for growth. Subcultures were made onto plates of SDA and incubated for 3-7 days at 25℃. Pure culture of fungal isolates were characterized and identified based on their cultural and morphological features such as type of soma, nature of hyphae, pseudo-mycelium and asexual reproductive spore as described by [22,23]. Suspension of the fungi was made using normal saline, a loopful of culture placed on a glass slide, overlaid with a cover slip and examined microscopically under high power using 40 x objectives. Sugar fermentation, assimilation and germ tube tests were further adopted for further characterization of yeasts.

## 2.4 Antibiotic Susceptibility Testing

*In vitro* susceptibility of the bacterial isolates to antibiotics was determined using disc-diffusion technique of [24]. Approximately, 0.1 ml of each bacterial isolates prepared directly from an overnight agar plate adjusted to 0.5 McFarland Standard was inoculated onto each of the Petri dishes containing Mueller-Hinton Agar. The antibiotic discs containing penicillin (Pen,10 µg), streptomycin (Stp,10 µg), iminipen (Imi,10 µg), ceftriaxone, (Cef, 30 µg), ceftazidime, (Cfp ,30 µg), cefotaxime (Cfo, 30 µg), ofloxacin (Ofl, 5 µg), levofloxacin (Lev, 5 µg) and moxifloxacin (Mox, 5 µg) (Oxoid, UK) were aseptically placed on the surfaces of the culture plates using a sterile forceps and gently pressed to ensure even contact. The plates were incubated at 37℃ for 18-24 hr. Inhibitory zones after incubation were observed and the diameters measured in millimeters using a ruler. The interpretation of the measurement as sensitive and resistant was made according to the manufacturer's standard zone size interpretative manual.

## 2.5 Determination of Multiple Antibiotic Resistance (MAR) Index

Multiple Antibiotic Resistance (MAR) index was determined using the formula MAR=*x/y,* where '*x*' was the number of antibiotics to which test isolate displayed resistance and '*y'* was the total number of antibiotics to which the test isolates has been evaluated for sensitivity [25]. Isolates that were resistant to ≥ 3 antibiotics were taken to be multiple antibiotic resistant [26].

## 2.6 Antifungal Drug Susceptibility Testing

*In vitro* susceptibility of the fungal isolates to antifungal drugs was determined using disc-diffusion technique. Zero point one (0.1) ml of the suspension of each fungal isolate was inoculated onto each of the Petri dishes containing MHA for *Candida* spp and SDA for other fungi [27]. The antifungal discs containing fluconazole (Flu, 25 µg), ketoconazole (Ket, 25 µg), voriconazole (Vor, 1 µg) and nystatin were aseptically placed on the agar plates. The plates were incubated at 37℃ for 24-48 hr for *Candida* spp and 25℃ for 48-72 hr for other fungal spp. Inhibitory zones after incubation were observed and measured in millimeters using a ruler. Results were interpreted as sensitive or resistant according to [27].

## 2.7 Detection of Deoxyribonuclease (DNase) Producing Micro-organisms

This test was carried out using DNase agar (Oxoid, UK). Spot inoculations were done onto to the surface of the DNase agar medium and incubated at 37℃ for 48 hr. After incubation, the growth on the surface of the agar was flooded with 1N HCl. The organisms that produced DNase enzymes, in sufficient quantity to hydrolyze DNA present with clear zones around the colonies [28].

## 2.8 Beta-lactamase Production Test

The production of β-Lactamase enzymes was determined by chromogenic cephalosporin method using nitrocephin (Oxoid, UK).

Nitrocephin is a chromogenic cephalosporin that changes colour from yellow to red with hydrolysis [28,29]. The colonies of the test bacteria were picked using wire loop and inoculated into sterile nutrient broth, incubated at 37℃ for 24 hr. Two drops of nitrocephin solution were added to each broth culture for colour change within 30 min. The β-lactamase production was inferred when the broth turned red within 30 min after addition of the reagent.

### 2.9 Haemolytic Assay of Bacteria

The haemolytic activities of the bacterial species was identified by the presence of clear (β-haemolysis) or greenish colouration (α-haemolysis) halos around the colonies on Columbia blood agar base (Oxoid, UK) supplemented with 5% sheep blood. The bacterial suspensions were streaked onto the blood agar plates and incubated for 24 hr at 37℃. Observations of the haemolytic zone around colonies after incubation were made and the type of haemolysis was recorded as *α*, *β* or γ.

### 2.10 Haemolytic Assay of *Candida* spp.

The *Candida* spp isolated from the middle ear were screened for haemolysin production using Sabourand Dextrose Agar (SDA) supplemented with sheep blood. About 0.1 ml of the *Candida* spp suspension was inoculated onto Petri dish containing the SDA supplemented with sheep blood. The culture plates were incubated at 37℃ for 48 hr. Observations of the haemolytic zone around colonies after incubation were made.

## 3. RESULTS

Out of the one hundred and seventy-six (176) middle-ear samples obtained from confirmed cases of COM, 84 (48.0%) were males and 92 (52.0%) were females resulting in an overall male to female ratio of 1:1.1. Of the 176 patients with COM, aged ≤ 10 years constituted 28 (15.6%), while those aged 11 – 20 yrs , 21 – 30yrs, 31 – 40 yrs , 41-50 yrs , 51-60 yrs, ≥ 61 yrs and Unspecified age (USP) were 32 (20.5%), 20 (11.6%), 24 (13.9%), 23 (12.8%), 19 (10.8%), 14 (8.2%) and 12 (6.5%), respectively (Table 1). The result showed that there was significant difference in the incidence of COM between the age groups ($P<0.05$, $X^2$: 4.67; df: 7).

On the basis of morphological and biochemical characteristics, a total of 263 bacterial isolates belonging to ten (10) genera were obtained from the middle ear samples of patients with COM. Of 263 culture smears obtained from COM, 119 (45.3%) were Gram positive and 144 (54.7%) were Gram negative bacteria. The most predominant bacterial isolates associated with COM were *Staphylococcus aureus* 65 (24.7%), followed by *Pseudomonas aeruginosa* 53 (20.1%). The frequencies of other bacterial isolates were as follows: *Proteus mirabilis* 22 (8.4%), *Streptococcus pneumoniae* 21 (7.9%), Coagulase negative *Staphylococcus* spp 19 (7.2%), *Klebsiella pneumoniae* 18(6.8%), *Escherichia coli* 17 (6.5%), *Proteus vulgaris* 10 (3.8%), *Serratia marcescens* 9 (3.4%), *Streptococcus pyogenes* 10 (3.8%), *Enterobacter* spp 8 (3.0%), *Morgenella morganii* 7 (2.7%), and *Bacillus substilis* 4 (1.5%) (Table 2)

Four fungal genera (*Aspergillus, Cryptococcus, Fusarium and Candida*) were isolated from the 176 samples collected from patients with COM (Table 3). *Aspergillus* was the predominant genus comprising of *Aspergillus niger* and *Aspergillus flavus* with percentage of occurrences of 29.2% and 6.3%, respectively. 22 (22.9%) of *Candida albicans* and 10 (10.4%) of other *Candida* species were isolated, while *Cryptococcus neoformans* and *Fusarium* spp. had 17 (17.7%) and 13 (13.5%), respectively (Table 3).

The distribution of bacterial isolates associated with COM in relation to age and sex is illustrated in Table 4. The data obtained showed that one hundred and twenty-two (122) bacteria were obtained from male patients with COM, while one hundred and forty-one (141) bacterial isolates were obtained from female patients. The most frequently bacteria isolated in both sexes was *S. aureus* with 29 (23.8%) of occurrence in male and 36 (25.5%) of occurrence in female, while the bacteria with the lowest frequency in both sexes was *B. substilis* with 2 (1.6%) and 2 (1.4%) occurrences in male and female patients, respectively. There was statistical difference at $P < 0.05$ in the occurrence of bacteria in relation to gender. The results also showed that the highest number of bacterial isolates was obtained among the age group 11-20 yrs and lowest among the age group ≥ 61yrs (Table 4). Forty-three (43) fungal isolates were obtained from the male patients with COM, while 53 fungal spp were isolated from the female (Table 5). The occurrences of fungal isolates associated with

COM in relation to sex were statistically different at ($P < 0.05$) (Table 5).

The results of haemolytic activities of the bacteria isolated from COM showed that 97 (36.9 %) and 31 (11.8%) of the isolates were *α* and *β* producers, respectively. Among the Gram positive bacteria obtained, *S. pneumoniae* showed the highest prevalence of partial haemolysis (α) of 15 (71.4%), while *S. pyogenes* showed the lowest with 1 (10.0%). Among the Gram negative bacteria *P. aeruginosa* showed the highest prevalence of partial haemolysis (α) with 18 (34.0%), while *M. morganii* showed the lowest with 2 (28.6%). Of 31 isolates that showed positivity for β haemolysis, 7 (70.0%) were *S. pyogenes* and 15 (23.1%) from *S. aureus*, while all *P. mirabilis, P. vulgaris, S. marcescens, Enterobacter* spp*. C. freundii* and *K. pneumoniae* showed no haemolysis (Table 6). The results showed that out of 71 DNase producing bacteria obtained from COM, 64 were Gram positive, while 7 were Gram negative. Of the 64 Gram positive bacteria producing DNase, *S. aureus* was the highest DNase producers, followed by *S. pyogenes* 4 (40.0%) and CoN *Staphylococcus* spp. 1 (5.3%). DNase production was also found in 7 (77.8%) *S. marcescens* (Table 6). Varied percentages of β-Lactamase production among the isolates were also obtained, ranging from 55.6% in *S. marcescens* to 28.6% in *M. morganii* (Table 6). The results of prevalence of haemolysin and DNase producing *Candida albicans* and other *Candida* spp isolated from COM are shown in Table 7.

The results of the antibiotic susceptibility patterns of the bacteria obtained from the swabbed samples of patients with COM showed that the two hundred and sixty-three (263) bacterial spp. exhibited varied degrees of sensitivities to the nine (9) antibiotics used (Table 8). The results showed that 143 (54.4%), 172 (65.4%) and 193 (73.4%) of the isolates were sensitive to penicillin, streptomycin and imipenem, respectively, while between 55.9% and 60.8% of the isolates were sensitive to ceftriaxone, cefotaxime and ceftazidime, 175 (66.5%) isolates were sensitive to ofloxacin, 204 (77.6%) were sensitive to levofloxacin and 198 (75.3%) were sensitive to Moxifloxacin. The results also showed that all the *B. substilis* isolated were sensitive to both levofloxacin and moxifloxacin, while between 44.4% to 62.5% of *Enterobacter* spp and *S. marcescens* were resistant to penicillin, ceftriaxone, cefotaxime and ceftazidime (Table 8). The multiple antibiotic resistance (MAR) indexes of the bacterial isolates are shown in Table 9. Overall, 243 (92.4%) of the bacterial isolates obtained from COM demonstrated a multi drug resistant phenotype (resistance to three or more antibiotics). Only 10 (3.8%) isolates were sensitive to all drugs, 10 (3.8%) were single drug resistant and 60 (22.8%) were resistant to 2 antibiotics. Of the 243 MDR bacteria, 47(19.3%) were resistant to three antibiotics, 136 (56.0%) were resistant to 4-8 antibiotics. The MDR *S. pneumoniae, B. substilis*, *S pyogenes* and CoN-*Staphylococcus aureus* had MAR indices ranging from 0.17 to 0.67 (Table 9). Fungal spp. isolated from COM were more susceptible to nystatin, voriconazole and fluconazole than ketoconazole. *C. albicans* and *A. nige*r were more sensitive to nystatin than other fungal spp., while 30.2-39.6% of the fungi were resistant to fluconazole and ketoconazole. Between 15.4% to 30.8% *Fusarium* spp. were resistant to fluconazole and voriconazole. Both *A. flavus* and *Candida* spp. were highly resistant to ketoconazole having 66.7% and 60.0%, respectively (Table 10)

## 4. DISCUSSION

Reports from different parts of the world showed the significance of bacterial and fungal as the causative agents of COM. The prevalence of this infection has been reported to be higher in developing countries compared to the advanced countries [30]. The results obtained in this study established the occurrence of COM infection in Akwa Ibom State. In this study, COM was found mostly among children and in young adults and similar results were obtained in some parts of Nigeria and India [31,32]. Analysis of age related prevalence of COM infections in this study showed that patients in the age group 11-20 yrs had a prevalence of 20.5% which was higher than that in the age group ≤ 10 yrs with a prevalence of 15.6%. Although, numerous studies have reported that males are at higher risk of otitis media than females, but several studies found no male-female disparity [33]. [34,35] reported that occurrence of otitis media was more in male than female and this is contrary to the results obtained in this study as 169 (48.1%) males and 183 (51.9%) of females had COM. The higher percentage of occurrence of *S. aureus* in females than males with otitis media observed in this study is in agreement with the earlier report of [36].

**Table 1. Age and gender distribution of chronic otitis media**

| Age (years) | Chronic otitis media | | Total |
|---|---|---|---|
| | Male | Female | |
| | No (%) | No (%) | No (%) |
| ≤ 10 | 15 (17.2) | 13 (14.2) | 28 (15.6) |
| 11-20 | 19 (21.9) | 17 (19.1) | 36 (20.5) |
| 21-30 | 8 (9.5) | 12 (13.7) | 20 (11.6) |
| 31-40 | 9 (11.2) | 15 (16.4) | 24 (13.9) |
| 41-50 | 12 (14.2) | 11 (11.5) | 23 (12.8) |
| 51-60 | 9 (11.2) | 10 (10.4) | 19 (10.8) |
| ≥ 61 | 6 (7.7) | 8 (8.7) | 14 (8.2) |
| USP | 6 (7.1) | 6 (6.0) | 12 (6.5) |
| Total | 84 (48.0) | 92 (52.0) | 176 (100) |

*USP: Unspecified; values in parenthesis are percentages*

It has been has shown that the most common bacterial and fungal species found in COM were *P. aeruginosa, S. aureus*, *P. mirabilis, K. pneumoniae, E. coli, Aspergillus* spp and *Candida* spp but these organisms vary in various geographical areas [37]. Results of types and percentages of bacterial isolates recovered from COM patients in this study were correlated with the results obtained by [38]. The frequency of *S. aureus* in the middle ear infections can be attributed to their ubiquitous nature and high carriage of resistant strains in the external auditory canal and upper respiratory tract [13]. *S. aureus* is an opportunistic pathogen found outside the human body, but when it gains entrance into the human body, it causes infection to tissues and mucus membranes and respiratory infections. Occurrence of *K. pneumoniae and Enterobacter* spp from middle ear of patients suffering from COM in this study is in conformity with the results obtained by [39,40]. Organisms like *E. coli* and *K. pneumoniae* become opportunistic pathogens in the middle ear when resistance is low. Aerobic bacteria such as *Pseudomonas aeruginosa* and *E. coli* were also isolated from COM in this study and this is in conformity with the reports of [2,41].

**Table 2. Frequency of distribution of bacterial isolates associated with chronic otitis media**

| Bacterial spp isolated | Number of occurrences | Percentage of occurrences |
|---|---|---|
| **Gram positive aerobes** | | |
| *Streptococcus pneumoniae* | 21 | 7.9 |
| *Streptococcus pyogenes* | 10 | 3.8 |
| *Staphylococcus aureus* | 65 | 24.7 |
| CoN *Staphylococcus* spp | 19 | 7.2 |
| *Bacillus substilis* | 4 | 1.5 |
| **Gram negative aerobes** | | |
| *Pseudomonas aeruginosa* | 53 | 20.1 |
| *Escherichia coli* | 17 | 6.5 |
| *Enterobacter* spp. | 8 | 3.0 |
| *Proteus mirabilis* | 22 | 8.4 |
| *Proteus vulgaris* | 10 | 3.8 |
| *Serratia marcescens* | 9 | 3.4 |
| *Morgenella morganii* | 7 | 2.7 |
| *Klebsiella pneumoniae* | 18 | 6.8 |
| Total | 263 | 100 |

*CoN : Coagulase negative*

**Table 3. Frequency of distribution of fungal isolates associated with chronic otitis media**

| Fungal spp. isolated | Number of occurrences | Percentage of occurrences |
|---|---|---|
| *Candida albicans* | 22 | 22.9 |
| *Aspergillus flavus* | 6 | 6.3 |
| *Aspergillus niger* | 28 | 29.2 |
| *Cryptococcus neoformans* | 17 | 17.7 |
| *Fusarium* spp | 13 | 13.5 |
| *Candida* spp. | 10 | 10.4 |
| Total | 96 | 100 |

**Table 4. Age and gender specific distribution of bacterial isolates among patients with chronic otitis media**

| Bacterial spp. isolated | Gender | | Age group | | | | | | | | Total 176 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Male No. (%) | Female No. (%) | ≤10 No. (%) | 11-20 No. (%) | 21-30 No. (%) | 31-40 No. (%) | 41-50 No. (%) | 51-60 No. (%) | ≥ 61 No. (%) | USP No. (%) | No. (%) |
| *S. pneumoniae* | 9 (7.3) | 12 (8.5) | 3 (14.2) | 4 (19.0) | 2 (9.5) | 4 (19.0) | 2 (9.5) | 4 (19.0) | 1 (4.8) | 1 (4.8) | 21 (7.9) |
| *S. pyogenes* | 5 (4.1) | 5 (3.5) | 2 (20.0) | 1 (10.0) | 1 (10.0) | 3 (30.0) | 1 (10.0) | 1 (10.0) | - | 1 (10.0) | 10 (3.8) |
| *S. aureus* | 29 (23.8) | 36 (25.5) | 8 (12.3) | 17 (26.1) | 8 (12.3) | 8 (12.3) | 10(15.4) | 5(7.7) | 4 (6.2) | 5 (7.7) | 65(24.7) |
| CoN*S.* spp | 8 (6.6) | 11 (7.8) | 3 (15.7) | 4 (21.1) | 2 (10.5) | 2 (10.5) | 2 (10.5) | 3 (15.7) | 1 (5.3) | 2 (10.5) | 19 (7.2) |
| *P. aeruginosa* | 25 (20.5) | 28(19.6) | 11 (20.7) | 9 (17.0) | 6 (11.3) | 5 (9.4) | 9 (17.0) | 6 (11.3) | 4 (7.5) | 3 (5.7) | 53 (20.1) |
| *E. coli* | 10 (8.2) | 7 (4.9) | 3 (17.6) | 5 (29.4) | 3 (17.6) | 2 (11.8) | 2 (11.8) | 1 (5.9) | 1 (5.9) | - | 17 (6.5) |
| *Enterobacter* spp | 3 (2.5) | 5 (3.2) | 1 (12.5) | 2 (25.0) | 1 (12.5) | 1 (12.5) | 1 (12.5) | 1 (12.5) | 1 (12.5) | - | 8 (3.0) |
| *P. mirabilis* | 10 (8.2) | 12 (8.5) | 3 (13.6) | 4 (18.2) | 2 (9.1) | 3 (13.6) | 2 (9.1) | 2 (9.1) | 4 (18.2) | 2 (9.1) | 22 (8.4) |
| *P. vulgaris* | 4 (3.3) | 6 (4.3) | 2 (20.0) | 2 (20.0) | 1 (10.0) | 2 (20.0) | 2 (20.0) | 1 (10.0) | - | - | 10 (3.8) |
| *S. marcescens* | 5(4.1) | 4 (2.8) | 1 (11.1) | 2 (22.2) | 1 (11.1) | 2 (22.2) | 1 (11.1) | 2 (22.2) | - | - | 9 (3.4) |
| *M. morganii* | 2 (1.6) | 5 (3.2) | 2 (28.6) | 2 (28.6) | - | 2 (28.6) | 1 (14.3) | - | - | - | 7 (2.7) |
| *B. substilis* | 2 (1.6) | 2 (1.4) | 1 (25.0) | 1 (25.0) | - | 2 (50.0) | - | - | - | - | 4 (1.5) |
| *K. pneumoniae.* | 10 (8.2) | 8 (5.7) | 3 (16.7) | 5 (27.8) | 2 (11.1) | 2 (11.1) | 1 (5.6) | 1 (5.6) | 3(16.7) | 1 (5.6) | 18 (6.8) |
| Total | 122 (100) | 141(100) | 43 (16.3) | 58 (22.1) | 29 (11.0) | 38 (14.4) | 34 (12.9) | 27(10.3) | 19 (7.2) | 15 (5.7) | 263 (100) |

*Key: USP: unspecified; CoNS: coagulase negative staphylococcus; values in parenthesis are percentages*

**Table 5. Age and gender specific distribution of fungi among patients with chronic otitis media**

| Fungal spp. isolated | Gender | | Age group | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Male No. (%) | Female No. (%) | ≤10 No. (%) | 11-20 No. (%) | 21-30 No. (%) | 31-40 No. (%) | 41-50 No. (%) | 51-60 No. (%) | ≥ 61 No. (%) | USP No. (%) | No. (%) |
| *C. albicans* | 10 (23.3) | 12(24.0) | 4(18.2) | 7 (31.8) | 1 (4.5) | 2 (9.1) | 2 (9.1) | 2 (9.1) | 2 (9.1) | 2 (9.1) | 22 (22.9) |
| *A. flavus* | 4 (9.3) | 2 (4.8) | 1(16.7) | 1 (16.7) | 2 (33.3) | 2 (33.3) | - | - | - | - | 6 (6.3) |
| *A. niger* | 11 (25.6) | 17(30.8) | 9(32.1) | 11 (39.3) | 3 (10.7) | 2 (7.1) | 1 (3.6) | 1 (3.6) | - | 1 (3.6) | 28 (29.2) |
| *C. neoformans* | 7(16.3) | 10(18.3) | 2(11.8) | 4 (23.5) | 1 (5.9) | 3 (17.6) | 3 (17.6) | 2 (11.8) | 1 (5.9) | 1 (5.9) | 17 (17.7) |
| *Fusarium* spp | 7(16.3) | 6(11.5) | 3(23.1) | 2 (15.4) | 3 (23.1) | 1 (7.7) | 2 (15.4) | 1 (7.7) | 1 (7.7) | - | 13 (13.5) |
| *Candida* spp. | 4 (9.13) | 6(10.6) | 2(20.0) | 2 (20.0) | 1 (10.0) | 1 (10.0) | 1 (10.0) | 2 (20.0) | 1 (20.0) | - | 10 (10.4) |
| Total | 43 (100) | 53(100) | 21(21.9) | 27 (28.1) | 11(11.5) | 11 (11.5) | 9 (9.4) | 8 (8.3) | 5 (5.2) | 4 (4.2) | 96 (100) |

*Key: USP: unspecified; values in parenthesis are percentages*

**Table 6. Prevalence of haemolysin, dnase and betalactamase producing bacteria in samples from chronic otitis media**

| Bacterial spp. | Haemolysin | | | | |
|---|---|---|---|---|---|
| | α | β | γ | DNase producers | β-lactamase producers |
| | No | No (%) | No (%) | No (%) | No (%) |
| *Streptococcus pneumoniae* | 21 | 15 (71.4) | 2 (9.5) | 4 (19.0) | 0 (0.0) | 9 (42.9) |
| *Streptococcus pyogenes* | 10 | 1 (10.0) | 7 (70.0) | 2 (20.0) | 4 (40.0) | 4 (40.0) |
| *Staphylococcus aureus* | 65 | 43 (66.2) | 15 (23.1) | 7 (10.8) | 59 (90.8) | 29 (44.6) |
| CoN *Staphylococcus* spp. | 19 | 11 (57.9) | 5 (26.3) | 3 (15.8) | 1 (5.3) | 8 (42.1) |
| *Pseudomonas aeruginosa* | 53 | 18 (34.0) | 0 (0.0) | 35 (66.0) | 0 (0.0) | 24 (45.3) |
| *Escherichia coli* | 17 | 6 (35.3) | 0 (0.0) | 11 (64.7) | 0 (0.0) | 5 (29.4) |
| *Enterobacter* spp. | 8 | 0 (0.0) | 0 (0.0) | 8 (100) | 0 (0.0) | 4 (50.0) |
| *Proteus mirabilis* | 22 | 0 (0.0) | 0 (0.0) | 22 (100) | 0 (0.0) | 7 (31.8) |
| *Proteus vulgaris* | 10 | 0 (0.0) | 0 (0.0) | 10 (100) | 0 (0.0) | 6 (60.0) |
| *Serratia marcescens* | 9 | 0 (0.0) | 0 (0.0) | 9 (100) | 7 (77.8) | 5 (55.6) |
| *Morgenella morganii* | 7 | 2 (28.6) | 0 (0.0) | 5 (71.4) | 0 (0.0) | 2 (28.6) |
| *Bacillus substilis* | 4 | 1 (25.0) | 2 (50.0) | 1 (25.0) | 0 (0.0) | 2 (50.0) |
| *Klebsiella pneumoniae* | 18 | 0 (0.0) | 0 (0.0) | 18 (100) | 0 (0.0) | 7 (38.8) |
| Total | 263 | 97 (36.9) | 31 (11.8) | 135 (51.3) | 71 (24.3) | 112 (42.6) |

*Keys: α: alpha; β: beta; γ: gamma; No: number of isolates; CoN: coagulase negative; values in parenthesis are percentages*

**Table 7. The prevalence of haemolysin and extracellular deoxyribonuclease (DNase) producing fungi isolated from chronic otitis media**

| Fungal spp | Number of Isolates | No/(%) of DNase producers | No/(%) of haemolysin producers |
|---|---|---|---|
| *Candida albicans* | 22 | 20 (90.9) | 10 (45.5) |
| *Aspergillus flavus* | 6 | - | - |
| *Aspergillus niger* | 28 | - | - |
| *Cryptococcus neoformans* | 17 | - | - |
| *Fusarium* spp | 13 | - | - |
| *Candida* spp. | 10 | 6 (60.0) | 2 (20.0) |
| Total | 96 | 26 (27.1) | 12 (12.5) |

*Values in parenthesis are percentages*

**Table 8. *In-vitro* antibiotic sensitivity spectrum of bacterial isolates from chronic otitis media**

| Bacterial spp | No. of isolates | Pen no. (%) | Stp no. (%) | Imi no. (%) | Cef no. (%) | Cfo no. (%) | Cfp no. (%) | Ofl no. (%) | Lev no. (%) | Mox no. (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| *S pneumoniae* | 21 | 11(52.4) | 15(71.4) | 18(85.7) | 12(57.1) | 12(57.1) | 13(61.9) | 15(71.4) | 18(85.7) | 17(81.1) |
| *S. pyogenes* | 10 | 5(50.0) | 5(50.0) | 7(70.0) | 5(50.0) | 6(60.0) | 6(60.0) | 6(60.0) | 7(70.0) | 7(70.0) |
| *S .aureus* | 65 | 35(53.8) | 46(70.8) | 50(76.9) | 36(55.4) | 34(52.3) | 36(55.4) | 43(66.2) | 46(70.8) | 44(67.7) |
| CON *Staphy.* .spp | 19 | 10(52.6) | 11(57.9) | 13(68.4) | 10(52.6) | 10(52.6) | 12(63.2) | 11(57.9) | 14(73.7) | 13(68.4) |
| *P. aeruginosa* | 53 | 28(52.8) | 36(67.9) | 45(84.9) | 27(50.1) | 28(52.8) | 28(52.8) | 34(64.2) | 45(84.9) | 47(88.7) |
| *Escherichia coli* | 17 | 11(64.7) | 10(58.8) | 10(58.8) | 12(70.6) | 12(70.6) | 13(76.4) | 11(64.7) | 13(76.4) | 12(70.6) |
| *Enterobacter* spp | 8 | 3(37.5) | 5(62.5) | 5(62.5) | 3(37.5) | 4(50.0) | 4(50.0) | 7(87.5) | 7(87.5) | 7(87.5) |
| *P. mirabilis* | 22 | 14(63.6) | 14(63.6) | 11(50.0) | 14(63.6) | 15(68.2) | 15(68.2) | 14(63.6) | 16(72.7) | 14(63.6) |
| *P. vulgaris* | 10 | 4(40.0) | 6(60.0) | 7(70.0) | 5(50.0) | 5(50.0) | 6(60.0) | 8(80.0) | 6(60.0) | 7(70.0) |
| *S marcescens* | 9 | 4(44.4) | 6(66.7) | 6(66.7) | 4(44.4) | 5(55.6) | 5(55.6) | 7(77.8) | 7(77.8) | 7(77.8) |
| *M. morganii* | 7 | 5(71.4) | 4(57.1) | 5(71.4) | 5(71.4) | 4(57.1) | 5(71.4) | 4(57.1) | 6(85.7) | 6(85.7) |
| *Bacillus substilis* | 4 | 2(50.0) | 3(75.0) | 3(75.0) | 2(50.0) | 3(75.0) | 2(50.0) | 2(50.0) | 4(100.0) | 4(100.0) |
| *K. pneumoniae* | 18 | 11(61.1) | 11(61.1) | 13(72.2) | 12(66.7) | 14(77.8) | 15(83.3) | 13(72.2) | 15(83.3) | 13(83.3) |
| Total | 263 | 143(54.4) | 172(65.4) | 193(73.4) | 147(55.9) | 152(57.8) | 160(60.8) | 175(66.5) | 204(77.6) | 198(75.3) |

*CoN: Coagulase negative staphylococcus; values in parenthesis are percentages; Pen: penicillin; Stp: streptomycin; Imi: iminipen; Cef: ceftriaxone; Cfo: cefotaxime; Cfp: ceftazidime; Ofl: ofloxacin; Lev: levofloxacin; Mox; moxifloxacin*

**Table 9. Multiple antibiotic resistance (MAR) Index of bacteria isolated from chronic otitis media**

| MAR index | Number / Percentage (%) | | | | | | | | | | | | | Total No. (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SP | BS | MM | SM | SPY | EN | PV | EC | KP | CoN | PM | PS | SA | |
| 0.0 | - | - | 1(14.3) | - | - | - | - | 1(5.9) | 2(11.1) | - | 1(4.5) | 3(5.7) | 2(3.1) | 10(3.8) |
| 0.08 | - | - | - | 1(11.1) | - | 1(12.5) | 1(10.0) | - | - | - | - | 4(7.5) | 3(4.6) | 10(3.8) |
| 0.17 | 5(23.8) | 1(25.0) | 2(28.6) | 2(22.2) | 2(20.0) | 2(25.0) | 2(20.0) | 2(11.8) | 3(16.7) | 2(10.5) | 2(9.1) | 19(35.8) | 16(24.6) | 60(22.8) |
| 0.25 | 7(33.3) | 1(25.0) | 1(14.3) | 1(11.1) | 1(10.0) | 2(25.0) | 2(20.0) | 5(29.4) | 3(16.7) | 1(5.3) | 4(18.2) | 6(11.3) | 13(20.0) | 47(17.9) |
| 0.33 | 4(19.0) | 1(25.0) | 1(14.3) | 3(33.3) | 2(20.0) | 1(12.5) | 3(30.0) | 3(17.6) | 5(27.8) | 6(31.6) | 5(22.7) | 6(11.3) | 14(21.5) | 54(20.5) |
| 0.42 | 1(4.8) | - | 1(14.3) | - | 2(20.0) | 1(12.5) | - | 3(17.6) | 2(11.1) | 6(31.6) | 5(22.7) | 3(5.7) | 6(9.2) | 30(11.4) |
| 0.50 | 3(14.3) | - | - | - | 1(10.0) | 1(12.5) | - | - | 1(5.6) | 1(5.3) | 1(4.5) | 4(7.5) | 4(6.2) | 16(6.1) |
| 0.58 | - | - | 1(14.3) | - | - | - | 2(20.0) | 3(17.6) | 1(5.6) | 2(10.5) | 1(4.5) | 5(9.4) | 4(6.2) | 19(7.2) |
| 0.67 | 1(4.8) | 1(25.0) | - | 2(22.2) | 2(20.0) | - | - | - | 1(5.6) | 1(5.3) | 3(13.6) | 3(5.7) | 3(4.6) | 17(6.5) |

*Keys : SP: S pneumoniae ; BS: B. substilis ; CF: C. freundii ; MM: M. morganii ; SM: S marcescens ; SPY: S. pyogènes ; EN: Enterobacter spp ; PV : P. vulgaris ; EC : E. coli ; KP: K. pneumoniae; CoN: coagulase negative staphylococcus spp. ; PM: P. mirabilis ; PS: P. aeruginosa ; SA: S. aureus*

**Table 10. *In-vitro* susceptibility of fungal spp. isolated from chronic otitis media to antifungal drugs**

| Fungal spp. | Number of isolates | Flu no. (%) | Nys no. (%) | Vor no. (%) | Ket no. (%) |
|---|---|---|---|---|---|
| *C. albicans* | 22 | 17(77.3) | 18(81.8) | 20(90.9) | 13(59.0) |
| *A. flavus* | 6 | 4(66.7) | 5(83.3) | 5(83.3) | 2(33.3) |
| *A. nige*r | 28 | 20(71.4) | 22(78.6) | 18(64.3) | 20(71.4) |
| *C. neoformans* | 17 | 12(70.6) | 12(70.6) | 10(58.8) | 11(64.7) |
| *Fusarium* spp. | 13 | 9(69.2) | 8(61.5) | 11(84.6) | 8(61.5) |
| *Candida* spp | 10 | 5(50.0) | 7(70.0) | 5(50.0) | 4(40.0) |
| Total | 96 | 67(69.8) | 72(75.0) | 69(71.9) | 58(60.4) |

*Keys values in parenthesis are percentages; Flu: fluconazole; Ket: ketoconazole; Vor: voriconazole; Nys: nystatin*

The occurrence of *A. niger, A. flavus, Candida* spp., *C. albicans, C. neoformans* and *Fusarium* spp in this research is an agreement with the reports of [42] where fungi such as *C. albicans, A. niger, Candida* spp. were implicated as causes of otitis media. The most frequently isolated mould in this study was *A. niger*, while the filamentous fungus such as *Fusarium* species was also on the increase. The highest occurrence of *A. niger* obtained in this study is in conformity with the results of [10]. Analysis of the MAR index of the bacteria isolated from COM showed that 92.4% of the bacteria had multiple antibiotic resistant index of 0.25 and above. MAR index higher than 0.2 has been said to be an Indication of isolates originating from an environment where antibiotics were often used [43].

The sensitivity of fungal species obtained from middle ear of patients with COM to fluconazole, voriconazole, ketoconazole and nystatin showed variable percentages of sensitivities. Azole has a broad spectrum of activity against *Candida* spp. and *Aspergillus* spp. Resistance of *C. albicans* and other *Candida* spp to ketoconazole in this study also correlates with the reports of [15].

The occurrence of 29.4% β-lactamase producing *e. Coli* in middle ear of patients with com in this study agrees with the reports of [44] that there is worldwide presence of β-lactamases in *E. Coli* isolates. The production of dnase by *S. aureus* in this study is in concordance with [45]. [14] has also revealed that one of the virulence factors produced by *S. aureus* is dnase. 16(84.2%) *S. marcescens* isolated from com produced dnase and this is in agreement with (ewing, 1986) who reported the production of dnase by *S. marcescens*. Dnase production was also found in fungal isolates such as *C. albicans* and *candida* spp and this agreed with [46] who reported that extracellular dnase production was a specific characteristic of genera of yeasts. Some strains isolated from com produced *β*-haemolysin with prevalence of 11.8%. Prevalence of *β*-haemolytic *S. aureus* strains in this study is in agreement with other research papers [47].

## 5. CONCLUSION

While the battle between man and microbes continue, starting with the defeat suffered by penicillin and other antibiotics. It is important and valuable to find compounds that potentiate antimicrobial activity against multidrug resistant organisms especially those associated with chronic otitis media.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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