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Investigating Biofilm Formation on Air-Conduction Hearing Aids and Implementing Effective Decontamination Methods

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Abstract:

A wide range of contaminating bacteria and fungi growing on hearing aid surfaces as well as ear canals of HA users. A total of 150 specimens (Hearing aid swabs) were collected of both genders and different ages and from patients who attended Ramadi Teaching Hospital and Ramadi Children's and Maternity Hospital, as well as institutes and schools for the deaf in Baghdad-Iraq. All specimens (Hearing aid swabs) were taken in the period from September and December of 2023.

All specimens were cultured on selective, and differential media for both bacteria and fungal (Blood agar, MacConkey agar , Chocolate agar, Mannitol Salt agar, Cetrimide agar, Sabouraud Dextrose agar, and Chrome agar), Microscopic Examination (Gram reaction, cell shape, and arrangement), Biochemical tests (Oxidase test, Catalase test, Coagulase test) and finally Vitek-2 compact system were used to confirm the species of all isolates. Sterilization methods of hearing aids by hydrogen peroxide. with 3% and 5%, Astic acid (5-10%) and Alcohol 70%. To identify specific genes, primers were created with the help of NCBI and supplied by Micro-Gene Company in Korea.

The results showed that 65% were urban and 35% were rural, The results distributed between gender with hearing aids as follow,for male (20.8% right, 26.7% left, 6.7% bilateral), while for female (16.7% right, 20.8% left, 8.3% bilateral) respectively. percentage of microbes in our results (bacteria and candida) as follow: 50% *S.aureus* ,20.5% *Ps.aureuginosa,* 10.5% *S.epidermidis,* 3% *E.coli,* 3.3% *Proteus spp,* 8.2% *K.pneumonia* and 4.5% *Candida spp*. Time plays an important role in the process of disinfection and elimination of microbes from hearing aids users. Alcohol 70% was more disinfectant in eliminate from microbes than hydrogen peroxide and acetic acid. *The femA, hly, Ps, Pr,* and *K1* genes were responsible for causing bacterial pathogenicity.

Keywords: *Hearing Aids, Sterilization methods,Gene detection*

Introduction:

Users of hearing aids (HA) frequently visit otolaryngologists complaining of persistent irritations in the ear canals caused by bacterial/fungal otitis externa, allergic contact dermatitis from the earmolds connecting the HA to the ear canal, or wax impaction (Chandrasekhar *et al*., 2019). The difficulties associated with ear canal discomfort, itching, and discharge can often make using HA challenging or even impossible (Nishiyama *et al*., 2021). Regardless of the benefits to their hearing, some users of HA quickly lose interest in using the devices, while others choose to stop using them altogether due to irritation from buildup of debris in the canal (Schwartz *et al*., 2017).

The most difficult situation arises from persistent bacterial or fungal otitis externa, in which case otolaryngologists may decide to fit aids by less intrusive means, like middle ear implants (Jin *et al*., 2022). Research has shown that a variety of pathogenic bacteria and fungi can grow on the surfaces of hearing aids and in the ear canals of individuals who use hearing aids (Nappier *et al*., 2020). The relationship between the growths of these microorganisms and the clinical condition of HA-affected canals, as well as whether or not they truly cause an infection of the external auditory canals, remain unclear (Dhingra *et al*.,2023).

Due to its cul-de-sac layout and capacity to retain moisture, the external auditory canal is more susceptible to the development of otitis externa because it fosters the growth of a variety of bacteria and fungi (Haider *et al*., 2022). It makes sense that the ear molds of the HA clog the canal, increasing the likelihood of moisture retention there and raising the risk of otitis externa in HA users (Bagatto *et al*., 2023). Researchers have shown that when a HA and its ear mold clog the canal, the surrounding air becomes even warmer, darker, and more humid. This shifts the cerumen's pH balance toward an alkaline pH and creates an environment that is ideal for the growth of microorganisms (Yesha, 2022).

Given the aforementioned difficulties, vented HA mold would be preferable to non-vented in that it allows the ear canal to breathe, which lowers the likelihood of moisture retention in the canal. But according to Patel *et al*. (2023), the ear mold's vent could allow for an unwanted sonic feed-back.

Depending on their virulence and the wearer's immune condition, the growth of these bacteria in the ear canal may irritate its lining and lead to ear discharge, swelling, and itching (Khan *et al*., 2022). The potential for contact allergy reaction of the ear canal lining to the materials of the ear moulds has been identified as significant cause of ear irritation in HA wearers, in addition to the irritation of the ear canals caused by the colonization of the canals by microorganisms in HA users (Zeise *et al*., 2021).

2. materials and methods

2.1 Samples Collection

A total of 150 specimens (hearing aid swabs) were collected from patients of all genders and ages who attended Ramadi Teaching Hospital, Ramadi Children's and Maternity Hospital, and deaf institutes and schools in Baghdad, Iraq. All specimens (hearing aid swabs) were taken between September and December 2023.

Laboratory diagnosis

All specimens were cultured on selective and differential media for both bacteria and fungal (Blood agar, MacConkey agar , Chocolate agar, Mannitol Salt agar, Cetrimide agar, Sabouraud Dextrose agar, and Chrome agar), Microscopic Examination (Gram reaction, cell shape, and arrangement), Biochemical tests (Oxidase test, Catalase test, Coagulase test) and finally Vitek-2 compact system were used to confirm the species of all isolates.

Sterilization methods of hearing aids

1- Hydrogen peroxide

Hydrogen peroxide with 3% and 5% was add to container containing warm water at a ratio of 1:10,and immersing hearing aids in the solution. Hearing aids was leave in the solution for 5 minutes, 10 minutes, and 15 minutes after this time, hearing aids was rinse with cold water and finally leaft hearing aids to dry.

2- Astic acid

A small spoonful of white vinegar (vinegar containing 5-10% acetic acid) was mixed with a cup of water. A soft, clean cloth was used to gently clean the hearing aids with this solution and finally leaft hearing aids to dry.

3- Alcohol 70%

Seven parts of ethyl alcohol (with a concentration of 99%) were mixed with 3 parts of water. A soft, clean cloth was used to gently clean the hearing aids with this solution and finally leaft hearing aids to completely dry.

DNA Extraction and Amplification Conditions

To identify specific genes, primers were created with the help of NCBI and supplied by Micro-Gene Company in Korea. The sequence of the primer pairs, amplification conditions and the amplicon size were mentioned in the table (1).

Table (1):Steps of PCR assays

Steps	Temperature (°C)	Time	Cycles
Initial Denaturation	95	5 min	1
Denaturation	95	30 sec	30
Annealing	59	30 sec	
Extension	72	30 sec	
Final extension	72	7 min	1

Results

Distribution of samples according to Residence

The results showed that 65% were urban and 35% were rural, as shown in figure (1)

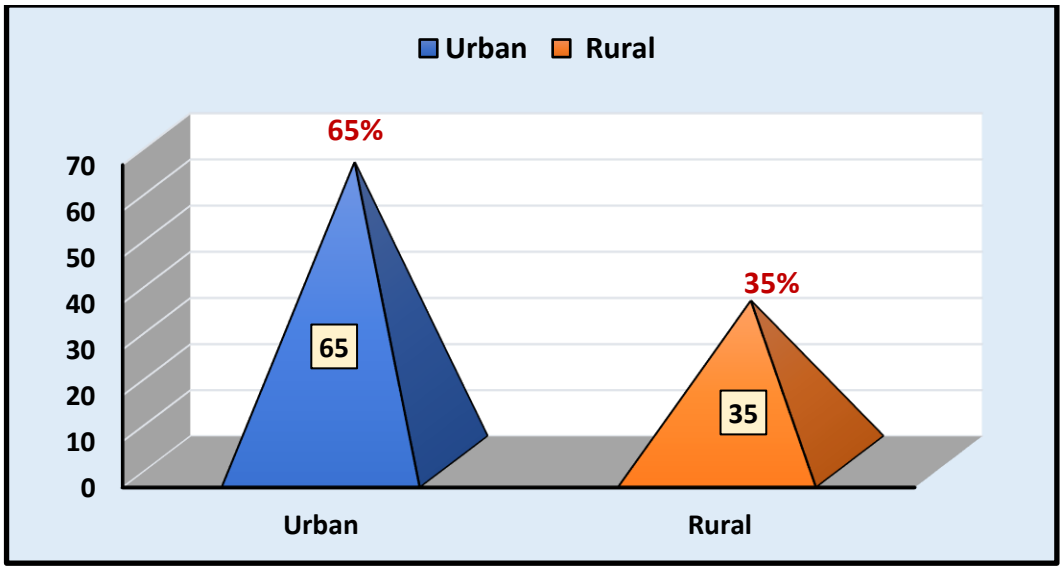

Figure (1): Distribution of samples according to Residence

The current results presented the percentage of hearing aids infected with microbes was higher in urban than rural. A study conducted by Agarwal and Devi,2017 showed urban was higher infection than rural, this results agree with the current study.

Also Sandhu *et al*. 2020 showed that infection more of urban patients (68.3%) as compared to patients from rural background (31.7%).

Demographics influence auditory settings and the use of hearing aid features. Younger urban people had the most diverse or challenging auditory settings and hearing aid feature activation, whereas older rural dwellers with hearing loss have the least diverse or demanding auditory environments and hearing aid feature activation (Jorgensen *et al*., 2023). Future research on real-world auditory environments and the

effectiveness of audiology interventions should take location into account when recruiting participants and interpreting results. Rural communities that practice self-cleaning and use home remedies to treat ear illnesses are more likely to develop otomycosis (Aremu *et al*., 2020).

Otomycosis is a prevalent problem in warm and humid climates, as well as in rural communities where people utilize traditional medicines for ear infections, which predispose them to the condition. Thus, educating people to reject such beliefs can help to alleviate the situation in such communities (Adhavan, 2020). Hearing loss has a significant impact on the quality of life, cognitive function, education, communication, workplace productivity, and social and emotional well-being of children and adults (Lieu *et al*., 2020).

Distribution of samples according to Gender

The results distributed between gender with hearing aids as follow,for male (20.8% right, 26.7% left, 6.7% bilateral), while for female (16.7% right, 20.8% left, 8.3% bilateral) respectively as shown in figure (2).

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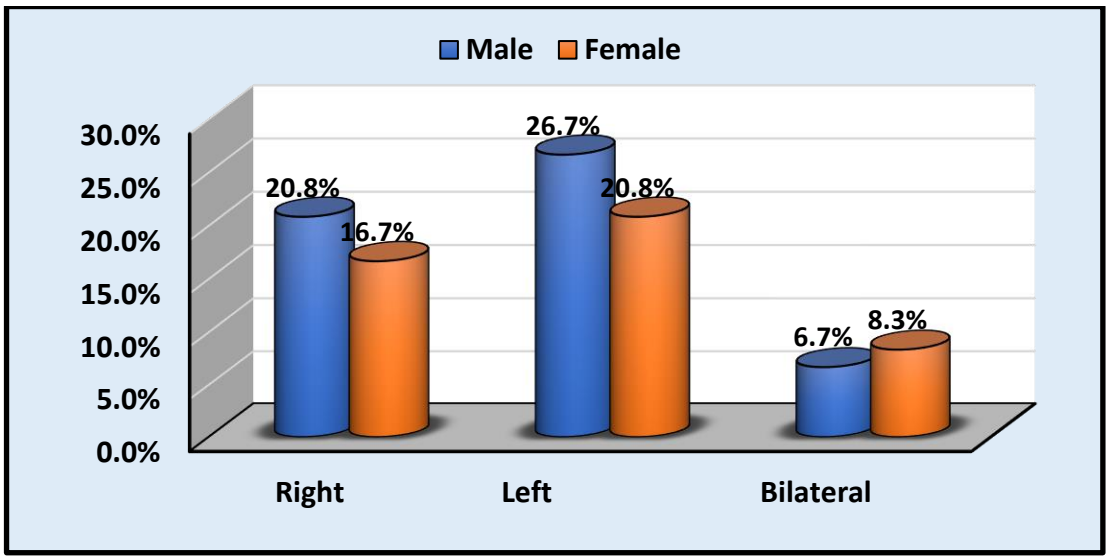

Figure (2): Distribution of samples according to Gender

The results shown in figure 2 showed that infected in males were higher in females,several results agree with the present results: Agha and Al-Delaimi,2021 was found in study that males 52.1% more infected than females and prevalence of infection was not significantly affected by gender. Which was in line with other studies conducted in Iraq (Aldhaher *et al*., 2018).

Tesei *et al*.,2022 found that among 350 study subjects, 199 were males and 151 were females, with a male to female ratio that is consistent with the results of the current study.

Male predominance could be attributed to lifestyle choices and environmental factors, which corroborate the occurrence of sex-based differences caused by relative genetic effects (Ferraz, 2023).

Sex and gender are increasingly recognized as important in medicine and research. Women tend to have higher immune responses to self and foreign antigens than men, resulting in gender disparities in autoimmunity and infectious illnesses (Dias *et al*., 2022). Males are more susceptible to bacterial infections than females, both in animals and in humans. At the same time, gender variations in health-seeking behavior, healthcare quality, and treatment adherence have been found (Enoksson *et al*., 2020).

Females have higher innate and adaptive immune responses than males, which allows for better pathogen clearance and response to vaccination, but also makes females more susceptible to inflammatory and autoimmune illnesses (Ray *et al*., 2020). Patient sex is a significant factor in health and disease, and infectious diseases are no exception. Biological sex (characterized by sex chromosomal complement, sex steroid hormones, and reproductive organs) has been linked to infection susceptibility, pathogenesis, immunological responses, clinical presentation, illness severity, and response to treatment and vaccination (Ferraz, 2023).Gender roles (socially created features) and social norms, on the other hand, might influence risk factors and infection exposure, as well as health-seeking behaviors and therapeutic decisions (Tesei *et al*.,2022).

Isolation and Identification of specimens

Identification of specimens were done by using culture media , Microscopic Examination, Biochemical tests and finally Vitek-2 compact system were used to confirm the species of all isolates and the results shown in figure (3) revealed that the percentage of microbes (bacteria and candida) as follow: 50% *S.aureus* ,20.5% *Ps.aureuginosa,* 10.5% *S.epidermidis,* 3% *E.coli,* 3.3% *Proteus spp,* 8.2% *K.pneumonia* and 4.5% *Candida spp*.

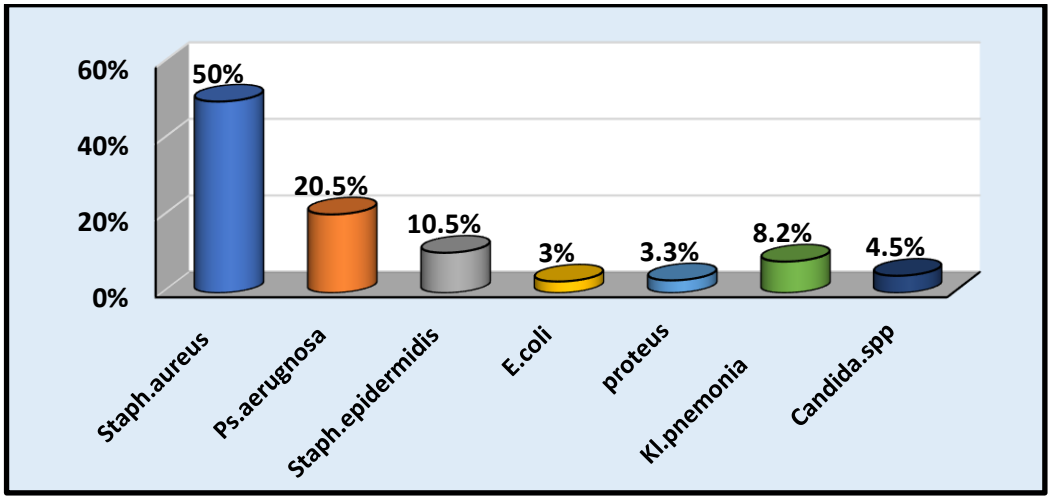

Figure (3): The percentage of microbes (bacteria and candida)

The results shown in figure 3 showed several pathogenic bacteria (gram positive and negative bacteria) and candida were isolated from hearing aids users, many studies agree with the current results.

Hailu *et al*. (2016) found that 296 (80.4%) of the 368 swab samples processed were culture positive, with 289 (97.6%) being bacteria and 7 (2.4%) being yeast cells, and that the predominant isolate was *Pseudomonas aeruginosa* (29.7%), followed by Staphylococcus aureus (26.3%) and Proteus spp. (21.9%). According to Getaneh *et al*., 2021, *Staphylococcus aureus, Proteus mirabilis, Proteus vulgaris, Escherichia coli,* and *Pseudomonas aeruginosa* are the leading causes of ear infections in hearing aid users. The presence of a large number of multidrug-resistant strains necessitates periodic and continuous antibiotic usage monitoring in the study area. Another study conducted by Novak et al., 2020, indicated that Staphylococcus aureus, *Escherichia coli* and *Acinetobacter* species which was taken from 41 hearing aid users were diagnosis.

Over 430 million people (~5% of the global population) suffer from debilitating hearing loss, affecting their quality of life (WHO, 2021). Hearing loss is more common in poorer nations, with middle ear illness (one of the leading causes of hearing impairment) having the highest incidence (Tamblay ET AL.,2023). As a result, documenting bacterial etiologies of ear infections and associated AST results is critical for preventing the multifaceted effects of ear infection and guiding empirical treatment in low-resource areas (Getaneh *et al*.,2021). Hearing aids have been identified as a possible mode of microbial transmission (Zwirzitz *et al*., 2020). The reported annual incidence of acute otitis externa in the general population ranges from 1:100 to 1:250, with a seasonal and regional variability (Sanyaolu *et al*.,2022). Using hearing aid ear molds increases the risk of developing otitis externa. Aside from the increased humidity caused by wearing a hearing aid ear mold, it has been proposed that the existence of polymicrobial flora in ear molds may be an etiological factor in the development of otitis externa in people. Bojanović *et al*. (2023) revealed that while some microbes are typical of the ear, others are unsanitary.

The effect of Detergents on hearing aids

Table 3 presents a comprehensive overview of measurements taken at different time intervals figure 4, offering mean values along with LSD0.05 values and corresponding P-values. After 5 minutes, the mean value of 0.948 ± 0.043, represented by the letter 'b,' suggests a statistically significant difference between groups, substantiated by the LSD0.05 value of 3.919 and a P-value of 0.049. As time progresses to 10 and 15 minutes, the mean values increase to 0.983 ± 0.020 and 0.995 ± 0.004, respectively, both marked with the letter 'a,' indicating significant changes. The note about significant differences between groups, denoted by 'S,' and the clarification that groups with different letters are statistically different**.**

Table 3. Comparison among different time.

Time	Mean	$LSD_{0.05}$ P value
After 5 min	0.948 ± 0.043 b	3.919 0.0490^{S}
After 10 min	0.983 ± 0.020 a	
After 15 min	0.995 ± 0.004 a	

S: Significant difference between groups.
Groups with different letters are statistically different.

Figure (4):Comparison among different time.

Table 4 provides a concise comparison of mean values for different groups, including acetic acid at 10%, acetic acid at 5%, hydrogen peroxide at 3%, hydrogen peroxide at 6%, and Alcohol at 70%. The mean values, expressed as mean ± standard deviation, indicate slight variations in the measured parameter among the groups. Acetic acid at 10% has the lowest mean value of 0.946 ± 0.043, followed by acetic acid at 5%, hydrogen peroxide at 3%, hydrogen peroxide at 6%, and Alcohol at 70%, with the highest mean of 0.996 ± 0.043. Notably, the $LSD_{0.05}$ is 3.394 which indicate the level of significance and since p value = 0.0304 is less than 0.05 it means that there is significant difference between groups. The letters “a”, “b” , and “c” are used to show the differences between groups figure 5.

Table 4. Comparison among different groups.

Groups	Mean	$LSD_{0.05}$ P value
hydrogen peroxide 3%	0.975 ± 0.043 b	3.394 0.0304^{S}
hydrogen peroxide 6%	0.995 ± 0.043 a	
acetic acid 5%	0.963± 0.043 b	
acetic acid 10%	0.946 ± 0.043 c	
Alcohol 70%	0.996 ± 0.043 a	

S: Nonsignificant difference between groups.
.groups with different letters are statistically different.

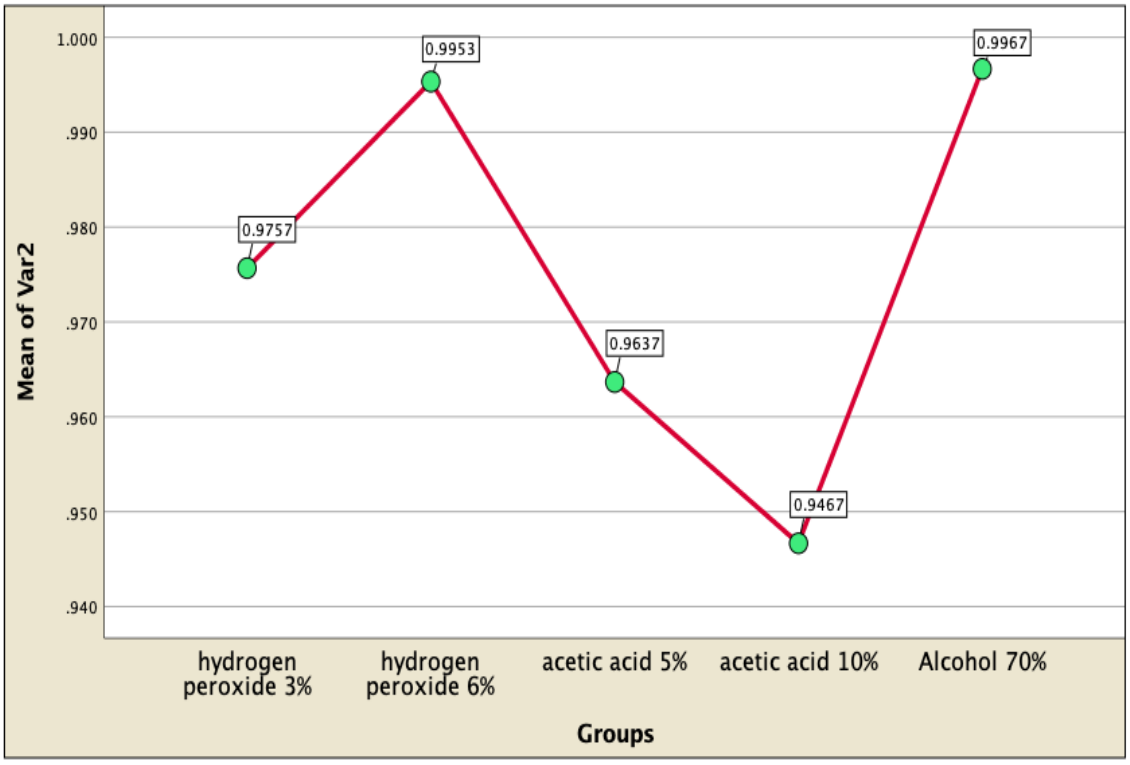

Figure (5):Comparison among Detergents.

Time plays an important role in the process of disinfection and elimination of microbes from hearing aids users, this study notice that sterile materials increase in disinfection as time increases, at 5 minutes the significant differences were 948 ± 0.043 and increased more at 15 minutes 0.995 ± 0.004. Hydrogen peroxide 6% and alcohol 70% recorded high level of disinfection follow hydrogen peroxide 3% and acetic acid 5% while acetic acid 10% was recorded low level.

Cleaning is described as the removal of filth from surfaces, whereas disinfection is the decrease of germs on surfaces, mostly by a microbiocidal effect, which may or may not be paired with mechanical removal (Artasensi *et al*., 2021). Biocides are chemical substances, mixtures, or microorganisms used to suppress hazardous organisms in ways other than physical or mechanical (Collet *et al*., 2022).

Multispecies biofilms are often thought to be less sensitive than monospecies biofilms; yet, some bacterial species within a complex biofilm have been demonstrated to protect susceptible ones (Yuan *et al*., 2022). Maillard and Centeleghe (2023) discovered that *Ps.aureuginosa, S.epidermidis*, and *E.coli* endoscope washer disinfector isolate, a strong EPS producer resistant to chlorine dioxide (0.03%), hydrogen peroxide (7.5%), and peracetic acid (2.25%), protected *S. aureus* from peracetic acid (0.35%) in a biofilm. Similarly, *Acinetobacter johnsonii* was demonstrated to protect *Salmonella* enterica subsp. enterica serovar Liverpool in a dual biofilm from benzalkonium chloride (300 mg/L). However, in this case, the decrease in susceptibility was coupled with a change in outer membrane lipid composition caused by the presence of S. aureus. Decreased biocide susceptibility to biocide has been described as a result of phenotypic bacterial adaptation within a biofilm. This is distinct from the impact of low biocide concentrations on bacterial phenotypic adaption within a biofilm, a phenomenon well documented by Boudjemaa *et al*. (2018).

According to the studies, high cell density in the biofilm community structure plays a vital role in biocide resistance (Zhu *et al*.,2021). Quorum sensing drives biofilm development, self-organization, and cell cooperation, but it also plays a role in other functions such as EPS synthesis, virulence factor expression, antimicrobial synthesis (including biosurfactant synthesis), and extracellular enzyme synthesis (Grobas *et al*., 2020). A sufficient concentration of QS molecules is required to induce a physiological response; in biofilms, QS molecules are expressed or accumulate due to high cell density (Buch *et al*.,2021).

Bacteria entrenched in biofilms are less sensitive than planktonic bacteria, which may explain the failure of surface disinfection, with bacteria remaining on surfaces following biocide exposure (Maillard and Centeleghe, 2023). In addition, one must consider the impact of biofilm maturity. Gene expression mediating various metabolic activity and resistance mechanisms has been found to vary as biofilms age (Sauer *et al*.,2022). Detached bacteria discharged from a biofilm have an intermediate sensitivity to biocides, falling somewhere between sessile and planktonic cell susceptibility; the resistance profile of detached bacteria may be related to the existence of EPS (Collet *et al*., 2022).

Molecular study of genes

The results shown in figure 6 showed the presence of (362bp) bands allowed for the detection of the hly gene in *E. coli* isolates , *K. Pneumoniae* serotypes were confirmed at molecular level by PCR analysis using specificprimerof magAgene specific for K1serotype with molecular weight 546 bp , the result of amplification femA gene was appeared as a clear band of (164 bp) as a clear band by electrophoresis of *S.aureus* ,also detection of *Ps.aureuginosa ps* (486) bp genes and *Proteus spp pr* (272).

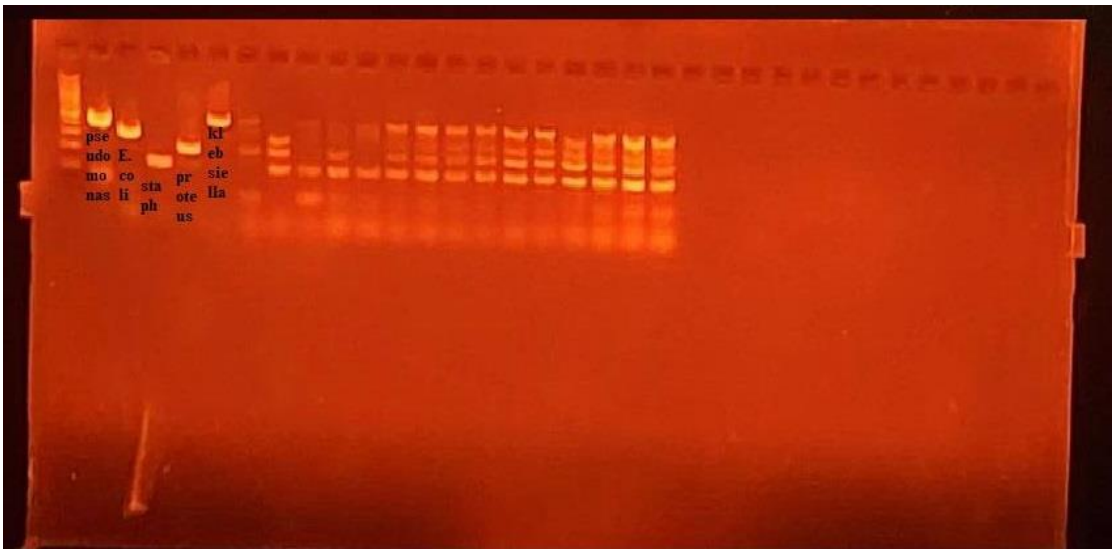

Figure (6):Detection genes of bacteria

Because of its toxicity, *E. coli* is a common cause of extraintestinal infections in health facilities around the world. To establish infection, the *E. coli* organism must first adhere to the host cell, which is accomplished via its surface adhesins (Sarowska *et al*., 2019). In Japan, *E. coli* isolates contained a greater percentage of hly-encoding genes (41.2%) (Deku *et al*., 2022). *HlyA* is a pore-forming exotoxin that lyses red blood cells and human renal epithelial cells by producing pores in them (Los *et al*., 2013). The siderophore system allows *E. coli* to use the iron released by lysed erythrocytes. Its generation and expression are determined by the availability of iron.
Qasim and Khalid (2022) examined 146 *K. Pneumoniae* and discovered that magA is unique to the *K. Pneumoniae* capsule serotype K1gene cluster, with all non-K1 strains being magA negative. As a result, PCR analysis for magA provides a quick and accurate technique for diagnosing *K. Pneumoniae* serotyping K1 isolates (Al-Jailawi *et al*., 2014). *K. pneumoniae* develops many virulence factors, such as lipopolysaccharides, fimbriae for adhesins, an antiphagocytic capsule (K antigen), and siderophores for iron acquisition from the host (Hiremath, 2018).The capsule is a vital virulence element for Klebsiella because it shields escaping bacteria from host destruction by phagocytosis while also stopping the host's immunological response. Qasim & Khalid (2022). The *MagA* gene is located within an operon exclusive to the serotype K1capsule cluster gene, regardless of origin (Walker and Miller, 2020). K1 and K2 are more harmful to humans than non-K1-K2 serotypes because of their polysaccharide capsule, which allows them to resist macrophage phagocytosis (Arato et al., 2021). Several studies have used femA as a marker for the identification of methicillin resistance. However, *femA* alone is not sufficient to provide methicillin resistance. According to studies, *fem* (factors necessary for methicillin resistance) or auxiliary genes like fem A/B/X, in addition to *mecA*, play a vital role in the expression of methicillin resistance (Fadhil and Mohammed, 2022).

Although finding the *femA* gene remains the gold standard for diagnosing methicillin resistance, it does not establish the existence of S. aureus, and there is no agreement on the molecular target that may be utilized to confirm the *S. aureus* species (Arifa et al., 2018). Molecular targets for identifying *S. aureus* species include constitutively expressed genes such femA and femB (Fisher and Mobashery, 2020). *Pseudomonas aeruginosa* produces numerous virulence factors that have been implicated in pathogenesis (Khuris *et al*.,2020). Antimicrobial resistance and virulence-associated genes were more common in biofilm-producing *Ps. aeruginosa* than in non-biofilm-producing strains (Kamali *et al*.,2020). Several factors contribute to increased antibiotic resistance, including reduced antibiotic diffusion through the biofilm exopolysaccharide matrix, decreased growth rates of biofilm bacteria, the formation of dormant persister cells, and the production of specific antibiotic resistance factors (Gajdács *et al*., 2021).

Conclusions:

The proliferation of microorganisms within the ear canal can potentially cause irritation of its lining and result in itching. The percentage of hearing aids infected with microbes was higher in urban than rural. Males were higher infected by microbes in hearing aids than females.The predominant bacteria were isolated from hearing aids users was *S. aureus* and *P. aeruginosa* . Time plays an important role in the process of disinfection and elimination of microbes from hearing aids users. Alcohol 70% was more disinfectant in eliminate from microbes than hydrogen peroxide and acetic acid. *The femA, hly, Ps, Pr,* and *K1* genes were responsible for causing bacterial pathogenicity.

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