



Research Article

Investigating the ability of bacteria isolated from various artificial respiratory systems to resist antibiotics and biofilms formation

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Abstract

Respiratory infections associated with ventilators represent a significant risk to individuals in intensive care units, particularly children under 3 years of age. 100 sample were collected Between October 2021 and February 2022, from Mosul hospitals (Ibn Al-Atheer Teaching Hospital, Ibn Sina Teaching Hospital, Al-Salam Hospital, and Al-Khansa Teaching Hospital) at ages 1 day to 3 years. A swab was taken from the water chamber of the artificial breathing devices, and the catheter head was collected, where the mucous material was cultured in three different mediums (blood agar, MacConkey agar, and Chocolate agar). 80 bacterial isolates were collected. The highest percentage was determined to be connected to Gr- bacteria, with 65 isolates 81.25%, and 15 isolates 18.75% being Gr+ bacteria. Using the Kirby-Bauer disc diffusion method, it was discovered that all isolates had great resistance to the antagonist's ampicillin and ceftriaxone, but high sensitivity to both Imipenem and Meropenem. The tube method was found to be more efficient than the Congo red agar method in the detection of biofilms. *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia* group, and *Pseudomonas mendocina* were biofilm-forming in both ways by up to 100%. The current investigation found that the tube approach produced 100% biofilm, but the Congo red agar method produced 41.25% biofilm.

Keywords: Biofilm, Tube method, Congo red agar method, Antibiotic, Pellicle

Introduction

Hospital-acquired infections are among the infections that may occur in 10% of hospitalized patients. They are more common in intensive care unit patients, and they are often associated with medical devices and equipment, especially respiratory devices, or after surgical interventions. Hospital-acquired pneumonia and bacteremia are among the most dangerous infections for patients (Jayaweera and Reyes, 2019). Respiratory device contamination causes hospital infection in two ways first: respiratory care equipment serves as a reservoir for microorganisms such as bacteria, fungi, and viruses, particularly Gr- bacteria. Second, contaminated devices can introduce bacteria into the airways if they are directly attached to the respiratory system (Kollef, 1993). Antibiotic resistance by hospital-acquired microbes in intensive care units is regarded substantial public health problem, as patients with acute and chronic disorders of very

young ages require lengthier hospital stays, the long stay period, Excessive usage of drugs, as well as their misuse and broad use, leads to antibiotic resistance in bacteria (Siddique et al., 2017).

The majority of bacteria colonizing respiratory organs are capable of forming biofilms, which are made up of a group of cells linked together by an exopolysaccharides matrix secreted by microorganisms and this facilitates adherence to medical surfaces, protection from the host immune system, and antimicrobial treatment (Mathur et al., 2006). The formation of biofilms in medical devices and natural environments increases their resistance to antibiotics, The creation of biofilms by bacteria that cause respiratory disorders, such as cystic fibrosis, may be serious, especially for patients hospitalized in intensive care units (Tirumalai and Prakash, 2012).

Materials and Methods

Swabs samples from the aqueous chamber of respiratory devices

90 swabs were collected from inside humidity water chamber for respiratory diseases devices (Ventilator, Suction machine, flow meter, Nebulizer). Swabs were obtained from the interior of the face mask and the surface of the Nasal cannula, which were attached to a flow meter to supply oxygen to the patient. The samples were placed in Glucose broth and incubated for 4 hours at 37°C (Jadhav et al., 2013; Leung et al., 2019; Shen et al., 2019).

Endotracheal tube samples

After taking 10 endotracheal tube samples from the patient, they were placed in a sterile container and transmitted directly to the laboratory. It chilled for 24 hours at 4°C in the refrigerator (Tille and Bailey, 2014).

Isolation methods

To obtain pure culture, samples in glucose broth medium were cultivated on MacConkey and blood agar Using the method of quadruple plotting and sterilization between each plotting. The incubation period was 24 hours at 37°C. In the case of catheters, the mucosal material contained within the catheters was cultured on blood and MacConkey agar for aerobic incubation at 37 °C for 24 hours and chocolate agar for anaerobic incubation with 5% CO₂ at 37 °C for 24 hours (Jadhav et al., 2013; Tille and Bailey, 2014).

Diagnosis

The bacteria were identified by growing them on several media, including EMB, Cetramide agar medium, and mannitol agar medium. It was observed the ability of isolates to grow on the above-mentioned media, and then their shape and color were noted, as well as their consumption of mannitol sugar on the mannitol agar medium. biochemical tests were carried out (Carroll et al., 2015). The API E20 system was used, and the Vitek-2 system was done to be certificated.

Antibiotic resistance

The sensitivity of the isolates under study was tested against antibiotics based on the Kirby-Bauer method. A single colony was taken from the young colonies at the age of 24 hours and placed in 5 cm³ of normal saline solution, The turbidity was set at 0.5 from a standard McFarland tube, which is equivalent to 1.5×10⁸ cells/ml, The inoculation was carried out using the smear method on the Muller Hinton Agar medium, then the plate was rotated several times and at an angle of 45° so that the agar was well impregnated with the bacterial suspension, The antibiotic disks were placed on agar medium with sterile forceps at identical distances apart, and the plates were incubated aerobically at 37°C for 18-24 hours.

The diameter of the inhibition zone was measured. The isolates were compared to standard tables based on the Clinical and Laboratory Standards Institute to determine if they are sensitive, moderately sensitive, or resistant to antibiotics (Tille and Bailey, 2014).

Investigation of biofilm formation

The production of biofilms in samples of respiratory devices and tools of the respiratory system was investigated using two methods: Tube and Congo red methods. Both approaches were dependent on the number of exogenous sugars produced by the bacteria when utilizing rich media such as brain heart infusion medium and Tryptic soya broth.

Tube method

To make a tryptic soya broth medium, dissolve 30 g in a liter of D.W. 1% glucose was added to sterilized broth via filtering. The isolates were grown after the medium was dispensed in glass test tubes. They were incubated at 37°C for 24 hours. The samples were emptied out and rinsed with phosphate buffer saline after incubation and left to dry. It was colored for 15 minutes using 0.1% crystal violet. The tubes were rinsed with deionized water and left in an inverted shape to dry. Polysaccharide production was determined by observing crystal violet precipitate on the bottom and walls of tubes. The lack of coloring indicates that bacteria are not producing biofilms (Ruchi et al., 2015; Bose et al. 2009).

Congo red agar method

A younger colony was cultivated on blood agar for 24 hours at 37°C a single colony was taken inoculated on Congo red agar and incubated aerobically at 37°C for 24 hours. After incubation, the colonies were examined to determine whether they were positive, with the appearance of a bright black color due to the secretion of gelatinous polysaccharides by the isolates, or negative with the appearance of a pink to red color due to the absence of gelatinous polysaccharides (Hassan et al., 2011; Ruchi et al., 2015).

Results and Discussion

Isolation and diagnosis

The results of the initial isolation of bacteria from respiratory devices for patients in intensive care for children aged 1 day to 3 years suffering from respiratory problems showed 80 bacterial isolates. The reason for the emergence of the high percentage of isolation from respiratory devices and tools may be attributed to sterilization inefficiency and neglect in sanitizing breathing devices. One of the reasons is a lack of attention to hygiene particularly in the intensive care unit, as well as the frequent usage of equipment from one patient to the next.

After phenotypic investigation of the isolates, it was found that the largest percentage was related to gram-negative bacteria as 65 isolates at 81.25%, while 15 isolates were obtained as gram-positive bacteria at a rate of 18.75%. Many researchers were referer that most infections resulting from artificial respiration are caused by Gram-negative bacteria, with multiple resistance to antibiotics, and Gram-negative bacteria are more pathogenic to humans than positive bacteria due to the nature of their complex wall and their multiple resistance to antibiotics (Borsi et al., 2018; Kentache et al., 2017).

Antibiotic resistance results

The development of resistance mechanisms by bacteria has an effect on patients in terms of the length of the recovery period from illness and the difficulty of recovery, as well as a rise in the death rate. So in our study to investigate the sensitivity and resistance to antibiotics depending on the fact that these tests are necessary for respiratory infections in children. the study comprised the use of 15 antibiotics from a variety of families. According to the Kirby-Bauer method, many isolates under examination showed multiple drug resistance (MDR). *S. maltophilia* exhibited high resistance to six antibiotics: Ampicillin, Ceftriaxone, Ceftazidime, Cefotaxime, Meropenem, and Imipenem. While two of the isolates *A. baumannii complex* exhibited strong resistance to Ampicillin, Ceftriaxone, Ceftazidime, Cefotaxime, and Meropenem. Furthermore, *B. cepacia group* showed resistance to three types of antibiotics: Ampicillin, Amikacin, and Azithromycin. the isolates of *S. maltophilia*, *A. baumannii complex*, and *B. cepacia group* can be classified as multi-resistance depending on the resistance towards three or more antibiotics belonging to a different group, The researchers (Gibb and Wong, 2021) pointed out the term MDR refers to a strain that is resistant to three or more different classes of antibiotics, and the reason for the resistance to antibiotics is due to the bacteria-harboring numerous resistance mechanisms. *A. baumannii* is one of the most common multidrug-resistant (MDR) bacteria found in hospitals worldwide. It is the cause of the highest rates of resistance in healthcare institutions, which leads to outbreaks of many diseases in the hospital environment. The reason for this is due to the ability of bacteria to adapt to a large extent to changes in the environment and the excessive use of antibiotics, all of these reasons led to the emergence of strains (MDR) (Kirtikliene et al., 2021; Al-Tamimi et al., 2022).

The investigation of resistance and sensitivity to antibiotics of enteric bacteria, including *Escherichia coli*, revealed that the isolates of the bacteria have variable resistance rates, with 90% resistance to Cefotaxime and Ampicillin. Ceftriaxone has a resistance rate of 85%. Some researchers attributed the resistance of *E. coli* to the cephalosporin family, such as Cefotaxime, Ceftazidime, and Ceftriaxone, to produce broad-spectrum beta-lactamase enzyme (ESBL), which is responsible for the hydrolysis of the antibiotic's beta-lactam ring. Furthermore, a mutation changes the shape of an enzyme this change leads to the interaction of the base material with

the enzyme or the binding of the antibiotic's active site (Mofolorunsho et al., 2021). The results revealed that the resistance rate of *Pseudomonas* isolates to antibiotics varied between isolates of the same species. *Pseudomonas aeruginosa* had the highest resistance to Cefotaxime at 87.87%, followed by ampicillin at 81.81%, and *Pseudomonas stutzeri* had 100% sensitivity to 5 antibiotics (Ceftazidime, Amikacin, Meropenem, Imipenem, and Ceftriaxone).

Resistance to Gram-negative bacteria has become prevalent and common in hospital-acquired infections. These bacteria have a substantial impact on the outcome of infection since they are linked to higher fatality rates and longer hospital stays. A considerable increase in the spread of ESBL was seen among bacteria causing nosocomial infections, particularly against third-generation of Cephalosporin such as (Cefotaxime, and Ceftriaxone), The majority belonged to the *Enterobacteriaceae*, *P. aeruginosa*, and *K. pneumoniae* families are resistant to the third generation of Cephalosporin (Zar & Cotton, 2002).

Biofilm formation results

Tube method

The positive result was investigated in terms of the formation of a violet layer in the walls and bottom of the inner tubes of the test tubes as in Fig.1.

We had 46.25% of the isolates with high and moderate ability to the production of biofilm, while 7.5% of the isolates showed low productivity. Our findings were consistent with those of (Hassan et al., 2011), as the tube approach yielded a percentage of 100% as a positive for biofilm. There was a variation in the percentages of productivity strength, as 19% strong (+++) the highest percentage of which was due to *P. aeruginosa*, 30% medium (++) which also had the highest percentage of *P. aeruginosa*, and 51% weak (+) of the biofilms.

According to the findings, *A. baumannii complex*, *E. coli*, and *P. aeruginosa* were made asuperficial layer on top of the culture media in the test tube, as illustrated in Fig. 2. The researcher Kentache et al. (2017) has indicated *A. baumannii* can colonize the upper surface of static fluids and develop biofilms in the upper part of the tube that is exposed to air and away from the tube's bottom called A pellicle. According to Karaca et al. (2013), *E. coli* and *P. aeruginosa* established robust assemblages in the bottom of the tube represented by biofilms, and a pellicle formed on the surface of the tube in contact with the air in the shape of a thin white ring.

Congo red agar

Based on the Congo red medium method of investigating biofilm formation, it was conducted that 41.25% of the total 80 isolates had the ability to form biofilms, as evidenced by the presence of colonies of bacteria developing in bright black

color as a positive result. On the contrary, the emergence of colonies in red or pink indicates a negative outcome of bacterial biofilm formation, as seen in Figure (3). Previous research had shown that the Congo red agar method is dependent on increasing polysaccharide formation by utilizing an enriching feeding medium. It employs the brain Heart Infusion with the addition of 5% sucrose, As well as the Congo red dye as a reagent showing the presence of polysaccharides, as the color changes in the last stages of incubation due to the presence of a specific metabolic product that interacts with the dye (Al-Omari, 2013).

Comparison results between the tube and Congo red agar

When the tube method and Congo red agar were used to test the production of biofilms, the tube method produced more positive findings than the Congo red method, as the percentage for the tube method was 100% compared to 41.25% for the Congo red agar. Our findings were consistent with those of (Furtuna et al, 2018) who investigated microbial contamination in medical equipment, particularly endotracheal and urine catheters, and claimed that the tube method is superior to the Congo red method, as the percentage in his study of the tube method was 88%, consisting of 32 isolates, whereas the percentage in his study of the red Congo method was 69%, consisting of 25 isolates. Also, the current study's findings agreed with those of (Al-Mola, 2021), who found that the tube method is more efficient in biofilm production than the Congo red agar method, which demonstrated a poor sensitivity to biofilm formation.

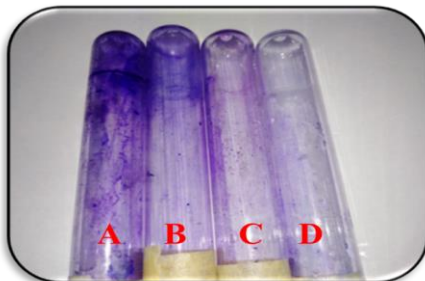


Fig. 1 Formation of biofilm by tube method: (A) strong, (B) medium, (C) weak, (D) non-forming of biofilm

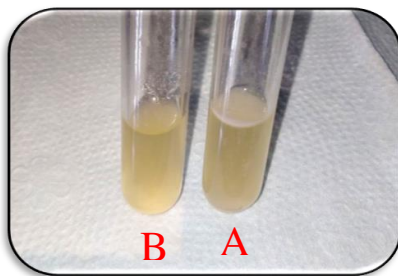


Fig. 2 Pellicle formation (A) pellicle-forming, (B) non-pellicle forming

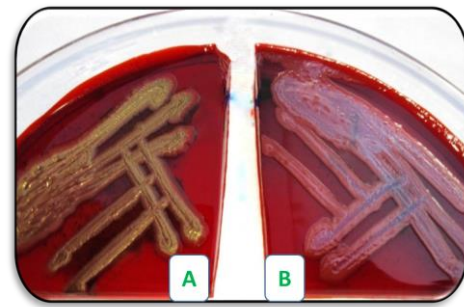


Fig. 3 Formation of biofilm by Congo red agar, (A) formed for biofilm in terms of bright black color, (B) non-forming for biofilm in terms of pink color for colonies

Conclusions

It was discovered that all isolates had great resistance to the antagonist's ampicillin and ceftriaxone, but high sensitivity to both Imipenem and Meropenem. The current investigation found that the tube method produced 100% biofilm formation, while the Congo red agar approach produced 41.25% biofilm formation. The tube method was found to be more efficient than the Congo red agar method in the production of biofilms. The isolates: *K. pneumoniae*, *S. maltophilia*, *B. cepacia* group, and *P. mendocina* were biofilm-forming in both methods.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

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