Evaluation of Quorum-Sensing, Antibiotics Resistance, and Biofilm Formation in Pathogenic Bacteria from the Hospital Environments

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Abstract

Background: Multidrug-resistant bacteria (MDR) often contaminate hospital environment and cause serious illnesses. Quorum Sensing (QS) regulates a variety of downstream cellular processes, including antibiotics resistance mechanisms and biofilm formation, and causes harm to the host. This study investigates antibacterial susceptibility and biofilm formation of pathogenic bacteria in hospital environment.

Methods: Hundred bacterial isolates were collected from various environments in the Medical City hospital. The antimicrobial susceptibility technique was evaluated through disk diffusion method. Next, biofilms formation was detected by the microliter plate assay. Finally, PCR was used to analyze the frequency of QS system genes.

Results: Current findings showed that the predominant isolates were Acinetobacter baumannii (34%), Escherichia coli (30%), Pseudomonas aeruginosa (19%), and Klebsiella pneumonia (17%). In general, significant resistance was found related to trimethoprim (88%), Augmentin (88%), and cefotaxime (72%). Among all isolates, 62% of sensitivity was related to ciprofloxacin. Biofilm had been formed by 39% of isolates. PCR results showed that the frequency of lasI and rhlI gene was 70% and 61%, respectively.

Conclusion: Current findings revealed that the hospital environment is a potential reservoir of MDR gram-negative pathogenic bacteria. Thus, we suggest that the health policymakers in Iraq must critically apply the guidelines and recommendations for monitoring the environments in the health sector.

Keywords: Antibiotics Footprint, Acinetobacter baumannii, Antibiotics Resistance, Quorum-Sensing, PCR.

1. Introduction

Nosocomial infections, also known as hospital-acquired infections, are serious global health concerns, mainly occurring during hospitalization and causing increased morbidity and mortality (Labi et al., 2019). A hospital environment is undoubtedly a great source of potentially pathogenic bacteria (Bouzada et al., 2010). It can be contaminated with bacterial pathogens, mainly Gram-negative (G-ve) rods such as Acinetobacter, Escherichia coli, Pseudomonas spp, Klebsiella sp, Shigella spp, Salmonella spp and Proteus spp, and Gram-positive (G+ve) cocci such as Staphylococcus aureus, Enterococcus and Streptococcus. Environmental surfaces serve as a reservoir for pathogenic bacteria (Otter et al., 2013). The development of nosocomial infection depends on a multifaceted relationship between the rate of contamination of the hospital environment, characteristics of the pathogen, and a susceptible host (Worku et al., 2018). Biofilm bacteria can share nutrients and are shielded from harmful environmental factors such as desiccation, antibiotics, and the immune system of a host body (Nirwati et al., 2019). In the hospital environment, biofilm-forming bacteria can associate with the ability to survive on surfaces, resist antibiotics, and face host defenses. Therefore, it contributes to cause chronic infections (Ali et al., 2019).

Quorum sensing are used by pathogenic bacteria to regulate gene expression. QS bacteria produce and release signals called autoinducers molecules (Häussler, 2010). Target genes regulate virulence factors, biofilm formation, and broad behaviors including swarming, swimming, twitching motility, and conjugation (Rutherford and Bassler, 2012). The most common QS system in G-ve bacteria involves the production of N-acylated homoserine lactones (AHLs) or autoinducer (Netotea et al., 2009). QS signaling will trigger biofilm formation, resulting in antimicrobial resistance of the pathogens, thereby increasing the therapeutic complexity of bacterial diseases (Jiang et al., 2019). In this regard, the main aim of
the current study is to identify the seriousness of a hospital environment as a potential reservoir of multidrug-resistant bacteria capable of infecting patients.

2. Materials and Methods

2.1. Bacterial isolates collection and identification

A total of one hundred bacterial isolates were collected from the surfaces, laundries, health care workers, and medical equipment in Medical City hospital in Baghdad, Iraq. Sterile swabs were used for the collected samples. These isolates were identified by routine biochemical tests and the Vitek 2 system.

2.2. Antibiotic Susceptibility test

The pattern of antibiotic susceptibility was done by the Kirby-Bauer method and interpreted according to the Clinical Laboratory Standard Institute guidelines (CLSI, 2020). Eight antibiotic discs were used in this study, which are Amikacin (AK, 30µg), Augmentin (AMG, 20 µg), Cefotaxime (CTX, 30µg), Cefepime (CPM, 30µg), Ceftriaxone (CTR, 30µg), Ciprofloxacin (CIP, 5µg), Trimethoprim (TMP, 5µg) and Piperacillin (PI, 30µg). These discs are provided from Bioanalyse in Turkey. The MICs of the isolates were determined by the diffusion method and interpreted as sensitive (S), intermediate (I), and resistant (R). The cut-off points were determined according to CLSI guidelines.

2.3. Biofilm formation assay

Microliter Plate Assay was performed for biofilm formation according to the method described by Babapour et al. (2016). First, 200 µl of bacterial suspension overnight culture (equivalent to 0.5 McFarland standard) was used to inoculate wells of a flat-bottom 96-well polystyrene microtiter plate (Coastar, USA), containing 180 µl of Brain Heart Infusion broth (Himedia, India) with 2% sucrose. After incubation at 37°C for 24hr., bacterial isolates were resistant to at least three different antimicrobials classes considered as MDR.

2.4. DNA extraction and gene amplification

DNA was extracted according to the manufacturer’s instructions of the DNA extraction kit (WizPrep™ gDNA Mini Kit, South Korea). Polymerase Chain Reaction was performed for amplification of lasR, rhlR, lasI and rhlI quorum sensing genes (See Table 1). Each PCR mix (25µl) was composed from 12.5µl of Go Taq® Green master mix, template DNA 5µl, forward & reverse primers (1µl for each), and 5.5µl of deionized nuclease–free water (Promega, USA).

PCR amplification conditions were as follows (all primers): initial denaturation at 95°C/5min followed by 36 cycles of 95°C/30sec, 59°C/1min and 72°C/1min, and a final extension at 72°C/10min (Cotar et al., 2010). The products were analyzed by 1% gel agarose (Promega, USA), containing 0.5 µg/mL of ethidium bromide and visualized under UV light.

Table 1: Primers oligonucleotide sequence and molecular size of PCR products

<table>
<thead>
<tr>
<th>Gene</th>
<th>Oligonucleotide</th>
<th>Product size bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>lasR</td>
<td>F: 5′-TGCGGATTTTCTGGGAACC-3′&lt;br&gt;R: 5′-CCGGCGAATATTTCCCCATATG-3′</td>
<td>401</td>
</tr>
<tr>
<td>lasI</td>
<td>F: 5′-TCGAGAGATGGAAATCGATG-3′&lt;br&gt;R: 5′-GCTGATGGCCGATCTTCAG-3′</td>
<td>402</td>
</tr>
<tr>
<td>rhlI</td>
<td>F: 5′-GAATTCGCTCTCGATCTGCT-3′&lt;br&gt;R: 5′-GGTCATAGGGCCGACTGTA-3′</td>
<td>182</td>
</tr>
<tr>
<td>rhlR</td>
<td>F: 5′-TCGATTTAATCGCCCTGCT-3′&lt;br&gt;R: 5′-TTCCAGAGCATCGCCCTGCT-3′</td>
<td>208</td>
</tr>
</tbody>
</table>

3. Results

The study aimed to identify the seriousness of hospitals environment as a potential reservoir of multidrug-resistant bacteria capable of infecting patients. The current finding showed that pathogenic bacteria heavily contaminate the surfaces of the hospitals. A hundred of bacterial Gram-negative isolates were identified. Among all isolates, A. baumannii were 34%, E. coli were 30%, P. aeruginosa were 19%, and K. pneumonia were 17%. The results of the antibiotics susceptibility test showed that most isolates were highly resistant against most antibiotics. The highest resistance was recorded for trimethoprim (88%), Augmentin (88%), and cefotaxime (72%). At the same time, ciprofloxacin (38%) was recorded as the most effective antibiotic against isolates. All isolates showed resistance to the rest of antibiotics ranging between (44%) and (66%), as shown in Figure 1.
The current obtained results showed that 39% of the environmental isolates were biofilm producers. The results recorded that four isolates with a percentage of 11.76% of A. baumannii isolates were strong biofilm producers. At the same time, most isolates were producers with weak biofilm with percentage of 66.66%. In general, K. pneumoniae was the high biofilm producers with percentage of 52.94%, followed by E. coli (43.33%), A. baumannii (35.29%), and P. aeruginosa (26.32%) (See Table 2).

Table 2: Adhesion patterns of isolates

<table>
<thead>
<tr>
<th>Isolates (Number)</th>
<th>Adhesion patterns</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strong</td>
<td>Moderate</td>
</tr>
<tr>
<td>A. baumannii (n=34)</td>
<td>4</td>
<td>Non former</td>
</tr>
<tr>
<td>E. coli (n=30)</td>
<td>Non former</td>
<td>5</td>
</tr>
<tr>
<td>P. aeruginosa (n=19)</td>
<td>Non former</td>
<td>Non former</td>
</tr>
<tr>
<td>K. pneumonia (n=17)</td>
<td>Non former</td>
<td>4</td>
</tr>
<tr>
<td>Total (%)</td>
<td>4 (10.26%)</td>
<td>9 (23.08%)</td>
</tr>
</tbody>
</table>

PCR analysis revealed that 70% of isolates carried the lasI gene, 61% of isolates carried the rhlI gene, 57% of isolates had the lasR gene, while 4% isolates carried the rhIR gene (See Figure 2 and Table 3). A. baumannii was the most bacterial isolate harboring quorum sensing genes, lasI, lasR, and rhlI genes found in 25 (73.52%) isolates, while 4 (11.76%) of isolates carried rhlR gene. The lasI, lasR, and rhlI genes were found in all isolates of P. aeruginosa (100%) and 13 (76.43%) isolates of K. pneumonia. Finally, 13 (43.33%) E. coli isolates contained lasI gene, and 4 (13.33%) isolates had rhlI genes.

Table 3: Number and percentage of QS genes presence in isolates

<table>
<thead>
<tr>
<th>Genes</th>
<th>baumannii (n=34)</th>
<th>P. aeruginosa (n=19)</th>
<th>K. pneumoniae (n=17)</th>
<th>E. coli (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>lasI</td>
<td>25 (73.52%)</td>
<td>19 (100%)</td>
<td>13 (76.43%)</td>
<td>13 (43.33%)</td>
</tr>
<tr>
<td>lasR</td>
<td>25 (73.52%)</td>
<td>19 (100%)</td>
<td>13 (76.43%)</td>
<td>Negative</td>
</tr>
<tr>
<td>rhlI</td>
<td>25 (73.52%)</td>
<td>19 (100%)</td>
<td>13 (76.43%)</td>
<td>Negative</td>
</tr>
<tr>
<td>rhIR</td>
<td>4 (11.76%)</td>
<td>Negative</td>
<td>Negative</td>
<td>4 (13.33%)</td>
</tr>
</tbody>
</table>

4. Discussion

Many studies showed that hospitals’ environment (surfaces, clothes, air, water, food, waste, and medical devices) harbor bacteria such as Staphylococcus, Enterococcus, A. baumannii, E. coli, P. aeruginosa, and K. pneumonia. Bacterial isolates in the hospital setting are characterized as MDR, not only for the irrational use of antibiotics but also for the presence of antibiotics residues in fluid effluents (Dougnon et al., 2020). Hospitals’ environments are characterized by heavy bacterial density (Ory et al., 2016). Antibiotic resistant bacteria pose a significant threat to public health in the hospitals’ environment (Osińska et al., 2017).

Our findings are consistent with several previous studies that showed various components of the hospitals’ environment could accommodate many pathogenic bacteria. According to Kim and co-workers, the areas around patients are generally contaminated by bacteria. The bacterial contamination on surfaces was supported by the formation of biofilms and prolonged survival in the environment (Kim et al., 1981; Talon, 1999; Bertrou et al., 2000).
Current findings revealed local isolates carrying one or more Qs genes. The Iraqi study conducted by Sallman et al. (2018) reported 82.53% isolates carrying lasI/lasR and rhlI/rhlR genes. Senturk et al. (2012) displayed that 77.7%, 88.8%, 66.6%, and 77.7% of isolates were positive for rhlR, rhlI, lasR, and lasI, respectively. QS involves generation, release and detection of extracellular signal molecules called auto-inducers (AI). It regulates behaviors requiring cells to synchronize in order to achieve successful results (Paluch et al., 2020). The QS system facilitates the bacterial population to grow and proliferate in an environment with effective intercellular communication (Subhadra et al., 2016). QS-controlled processes include antibiotic resistance, biofilm formation and virulence (Paluch et al., 2020).

Antibiotic-resistant A. baumannii has been represented as one of the most problematic hospitals acquired bacteria. A. baumannii can colonize in the hospital setting, and constitutes a significant problem in intensive care units (Espinal et al., 2012). A. baumannii was isolated from 11% (7/64) of air samples. Hospitals and healthcare settings are regarded as reservoirs of Pseudomonas spp isolates, which are a worldwide health concern due to the increasing development of MDR isolate (Alhusseini et al., 2019). Several therapeutic challenges exist with MDR P. aeruginosa due to the limit of effective treatment strategies (Aloush et al., 2006). The presence of pathogenic bacteria in the hospitals’ environment poses a significant risk to health. K. pneumoniae is recognized as an urgent threat to human health because of the emergence of MDR isolates associated with hospital outbreaks and hyper-virulent strains associated with severe community-acquired infections (Holt et al., 2015). Recorded hospital settings showed the highest percentage of 23% of extended-spectrum β-lactamase producing K. pneumonia (Chaudhry et al., 2019). Biofilm becomes a significant problem in health care (Dewasthale et al., 2018). Bacteria in a biofilm are a protective mechanism to survive in harsh conditions. These bacteria become more resistant to antibiotics; therefore, this biofilm structure represents an important virulence factor (Espinal et al., 2012).

Antibiotics resistance in biofilms is complex and results from contributions of intrinsic, acquired, and adaptive mechanisms. Most notably, biofilm specific features such as the differential expression of multiple gene networks, extracellular matrix, and the metabolic heterogeneity of subpopulations within a biofilm colony are significant contributors to antibiotic resistance (Taylor et al., 2014). P. aeruginosa and K. pneumoniae exhibited strong biofilm-forming ability on hospital clinical laboratory surfaces. Klebsiella spp. was found to persist on dry inanimate surfaces between 2 Hr. to 30 months, while the persistence of P. aeruginosa was 6 Hr. to 16 months (Chen et al., 2020).

5. Conclusion

The extracted findings from this study reported the prevalence of gram-negative pathogenic isolates in the hospitals’ environment. So, appropriate measures could help reduce pollutants in the hospitals’ environment and reduce related serious illnesses. As a result, the current findings recommend the routine screening and disinfection of the hospitals’ environment to prevent contamination.

6. Author’s contributions


7. Ethical approval

The authors do not see any ethical issues that may arise after the publication of this manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest in the publication.

References


