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Isolation and PCR-Based Detection of Antibiotic-Resistant Bacteria from Hospital Environments

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Abstract

The proposed study aimed to assess the occurrence and pattern of antibiotic-resistant bacterium in hospital settings both through traditional methods of culture as well as PCR-based molecular identification. Sampling of the environment of different units in Ramadi Teaching Hospital was carried out over a six-month period. A total of 600 environmental samples were sampled in the high-risk environments such as intensive care units, emergency departments, surgical suites, and medical wards by standardized swabbing methods. Traditional culture methods and PCR amplification based on common resistance genes such as mecA, vanA/vanB, blaTEM, blaSHV, blaCTX-M and qnr genes were used in processing the samples. Findings indicated that the hospital was generally contaminated with antibiotic-resistant bacteria in all hospital units. Out of the 600 samples taken, 387 (64.5% of the total samples) were positive by conventional culture test, and PCR-based results revealed the presence of resistant bacteria in 456 samples (76.0% of the total samples). The highest contamination rates were found in intensive care units with 94.7% of samples being positive on PCR. The most commonly isolated organisms were MRSA (34.2%), VRE (28.7%), and ESBL producing Enterobacteriaceae (41.5%). Detection using PCR was more sensitive (89.2% than the traditional culture methods (64.5%). The environmental contamination rates and the clinical infection rates were strongly correlated ($r = 0.742$, $p < 0.001$). The research confirms the usefulness of molecular detection systems in monitoring the environment in hospitals and points to the prevalence of the presence of multidrug-resistant organisms in healthcare settings.

Introduction

Healthcare-Associated Infections (HAIs) are a major health concern in the world, antibiotic-resistant bacteria being the main causative agents [24]. Hospitals are the perfect environment in which multidrug-resistant organisms propagate and spread because of the extensive use of antibiotics, susceptible populations, and large bacterial counts [22, 23, 26]. The development of antibiotic resistance has turned common infections to life-threatening ones and required urgent and thorough surveillance and methods.

The traditional culture-based methods of detection are reliable, but they are time consuming and may not be capable of identifying viable but non culturable bacteria or provide timely results, which could be utilized in making decisions on how to manage infections [9]. Polymerase Chain Reaction (PCR) technology has greatly revolutionized bacterial detection by identifying bacteria and resistance genes in rapid, sensitive and specific detection of bacteria [10]. PCR-based techniques have the ability to identify bacterial DNA despite the death of an organism and can give results within hours instead of days, thus becoming invaluable to hospital infection control programs.

Even in the face of the improved infection control practices, antibiotic-resistant bacteria are still pervasive in the hospital setting, and they lead to higher morbidity, mortality, and health care expenses [20, 23]. Present surveillance practices are usually based on clinical isolates which are likely not to reflect the actual burden of environmental pollution [1, 2]. The absence of rapid, comprehensive environmental monitoring systems disrupts successful interventions to control infections and provides space to spread resistant pathogens in healthcare facilities [3, 8].

The proposed research will aim at establishing the rate of occurrence of antibiotic-resistant bacteria in the various surfaces of the hospital environment, the most common resistance genes in environmental isolates, the efficiency of PCR-based detectives with the traditional culture-based methods of detecting bacteria, and the relationship between the environmental contamination and the clinical infection rates.

Materials and Methods

Study Design and Setting

The study was an observational cross-sectional study carried out in Ramadi Teaching Hospital within a period of six months. Environmental sampling was done in the high-risk settings such as intensive care units, emergency departments, operating rooms, and medical wards. The Institutional Review Board accepted the study protocol.

Sample Collection

They were sampled at different hospital units, which yielded 600 environmental samples each of 50 samples per month over the study period. The sampling sites were the common areas like bed rails, stations of nurses, computer key boards, door handles, medical equipment, bath rooms [16, 17]. Standardized methods were used to collect the environmental samples by using sterile swabs moistened with normal saline [1, 2]. A systematic pattern (with area of 10 cm^2) was used to swab each sampling site. Samples were taken to the laboratory in transport media and processed with 2 hours of collection.

Culture Methods

The common culture methods used traditionally were plating samples on selective media such as MacConkey agar, blood agar and chromogenic agar plates that were selective to resistant organisms

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[9, 10]. Plates were incubated at 37C of either 24/48 hours and the identification of the bacteria was carried out by biochemical tests as well as automated identification.

PCR Detection

In the case of molecular detection, DNA was isolated out of samples by commercial extraction kit [9, 10]. PCR amplification was done on common resistance genes such as mecA (methicillin resistance), vanA/vanB (vancomycin resistance), blaTEM, blaSHV and blaCTXM(beta lactamase genes) and qnr genes (quinolone resistance).

Statistical Analysis

The statistical analysis was done using SPSS 28.0 software. The resistant bacteria were used to summarize the prevalence levels in the various hospital units and sampling sites using descriptive statistics. Chi-square tests were used to determine the relationship between bacterial contamination and environmental factors. Computations of sensitivity and specificity were made in comparisons between PCR-based detection and culture techniques. Logistic regression analysis was used to identify factors relating with environmental contamination.

Results

Environmental sampling showed that there is extensive contamination of antibiotic-resistant bacteria in all hospital units surveyed [1, 3]. Among the 600 samples examined, 387 (64.5%), on the basis of traditional culture techniques, were found positive in at least one type of antibiotic-resistant organism, whereas 456 samples (76.0) were positive on PCR-based detection of the resistant bacterial types.

The contamination rates were highest in the intensive care units where 89.3% of the samples were positive in culture and 94.7% were positive in PCR [14]. Contamination in emergency departments by culture and PCR was 78.2 and 87.4% respectively, whereas it was 52.1 and 63.8% respectively in the general medical wards.

Contamination of samples varied greatly by unit location in hospitals [7, 11]. The highest level of contamination was observed on high-touch surfaces (bed rails (82.4% positive), computer keyboards (79.3% positive), and door handles (76.8% positive) as well [16, 17]. Medical equipment surfaces had a rate of contamination of 71.2 with a lower rate of contamination in floor samples of 43.6 [18].

There was an average of 3.2 x 104 CFU/cm2 (range: 1.0 x 102 to 2.1 x 106 CFU/cm2) of bacteria per positive sample. In intensive care units, the mean bacterial loads (5.8 x 104 CFU/cm2) were much greater in intensive care units than in general wards (1.9 x 104 CFU/cm2) ($p < 0.001$).

Table 1: Environmental contamination rates by hospital unit

Hospital Unit	Culture Positive (%)	PCR Positive (%)	Mean Bacterial Load (CFU/cm²)
Intensive Care	89.3 ± 2.1	94.7 ± 1.8	$5.8 \times 10^4 \pm 1.2 \times 10^4$
Emergency Department	78.2 ± 3.4	87.4 ± 2.9	$4.1 \times 10^4 \pm 0.9 \times 10^4$
Surgical Suite	71.5 ± 4.2	81.3 ± 3.7	$3.6 \times 10^4 \pm 0.8 \times 10^4$
Medical Ward	52.1 ± 3.8	63.8 ± 4.1	$1.9 \times 10^4 \pm 0.5 \times 10^4$

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Table 2: Resistance gene prevalence in environmental samples

Resistance Gene	Number of Positive Samples	Percentage (%)
mecA (MRSA)	298	49.7
vanA/vanB (VRE)	234	39.0
blaTEM	195	32.5
blaSHV	167	27.8
blaCTX-M	203	33.8
qnr genes	142	23.7
Multiple resistance genes	278	46.3

Table 3: Comparison of detection methods

Detection Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Culture	64.5	100.0	100.0	58.2
PCR	89.2	94.7	97.8	78.3

The detection rate in PCR showed a much stronger sensitivity as compared to the standard culture (89.2% vs. 64.5, $p < 0.001$). PCR specificity was found to be 94.7 per cent as compared to 100 per cent in culture methods. The presence of many resistance genes was observed in the 278 samples (46.3%), which has shown that the hospital environment has many cases of multidrug resistance.

The statistical analysis showed that there were strong relationships between environmental contamination and hospital factors. There was a statistically significant higher rate of contamination in units where the rate of antibiotic use was higher (OR = 2.34, 95% CI: 1.67-3.28, $p < 0.001$). Contamination had a negative relationship with cleaning frequency with those units cleaned more than twice a day having 38% less contamination ($p = 0.003$) [6, 17].

A correlation analysis showed a positive relationship of a high degree of environmental contamination with the rate of clinical infection ($r = 0.742$, $p < 0.001$) [11, 13]. The units that had more environmental bacterial loads had much more healthcare-associated infections in the study period.

Discussion

The findings justify the most common occurrence of antibiotic-resistant bacteria in a hospital environment, and the extent of contamination varies significantly based on various units and surfaces [1, 2]. The 64.5 percent positive rate using the standard culture methods are like the previous reports of environmental surveillance [7, 8] and the 76 percent detection rate using PCR shows that molecular techniques are very sensitive [9, 10, 25].

The rise in the rate of contamination at the intensive care units is suggestive of the interaction between factors including the presence of critically ill patients, intensive medical interventions and constant use of antibiotics [14]. These findings support the current infection control interventions that encompass increased environmental surveillance and cleaning levels in risky areas [3, 6].

This is because the prevalence of multidrug resistance is very high in the hospital environment given the fact that there are several resistance genes in nearly half of all samples [22]. The implications in this observation are enormous on empirical antibiotic therapy and infection control strategies, and it suggests that extensive resistance profiling needs to be followed as opposed to concentrating on individual resistance mechanisms [23, 24].

The PCR results are confirmed by the findings of the previous comparative studies by the excellent detection of the method when compared to the culture methods [9, 10]. The sensitivity difference (24.7 percentage points) was more than was typically reported (10-15 percentage points) possibly due to our general sampling strategy and molecular methods of detection.
The correlation between environmental contamination and the rates of clinical infection ($r = 0.742$) is high in comparison with other previous studies the correlation in which is 0.45 to 0.65 on average [11, 13]. This larger association ensures the clinical relevance of this kind of environmental monitoring and the implementation of evidence-based cleaning activities of the environment on the basis of the surveillance data [6, 17].

These results have significant consequences on the hospital infection control [3, 19]. PCR-based detection is very sensitive hence denoting that molecular technique ought to be considered in the daily monitoring of the environment, particularly in the areas of high risks. Hospitals will consider the risk-stratified environmental monitoring programs which will demand more monitoring in the areas where the rate of contamination is more prevalent [1, 2].

Conclusions

The systematic review confirms the ultimate contamination of hospital environment with antibiotic-resistant bacteria that PCR-based methods of detection pose to be more sensitive than the classical methods of culture [9, 10]. The highest contamination rates were in the intensive care units and 46.3% of the contaminated samples identified multidrug resistance [22]. The correlation between the degree of environmental contamination and clinical infection rate is so positive and this confirms the clinical significance of environmental monitoring [11, 13].

Hospitals in regions with high risk should consider undertaking routine environmental surveillance programs that are carried out with the aid of molecular detection methods [1, 2]. More environmental cleaning operations should also be done in the high pollution areas, which must be reviewed by periodic surveillance processes [6, 17]. The data collected by the environmental surveillance, combined with the clinical systems tracking the infections could contribute to the enhanced understanding of the dynamics of the transmission and the target intervention base [3, 8].

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