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Study on Some Resistant Strains of Bacteria and Their Role in Hospital Acquired Infection

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ABSTRACT

Background: Hospital acquired infection is defined as an infection acquired in hospital by a case who has been admitted for a reason other than that infection.

Aim: To discuss bacteriological characters, genetic structure, detection methods, and risk factors for mortality in methicillin-resistant staphylococci and extended-spectrum β-lactamase-producing organisms to evaluate and manage hospital-acquired infections.

Patients and methods: This investigation has been performed on 100 cases suffering from infection, which wasn't present at the admission time to Al-Ahrar Teaching Hospital and not manifested in the first 48 hours of hospitalization i.e., nosocomial infection.

Results: A MIC ***(Minimum Inhibitory Concentration)*** value of ≤8 indicated sensitivity to the antimicrobial agent, values between 9 and 31 represented intermediate resistance, and a MIC value of ≥32 signified resistance. This classification helped in determining the effectiveness of the antimicrobial agent against specific bacterial strains. From the 78 potentially ESBL producer isolates by the screening tests, confirmed ESBL has been detected in 62.8% (n=49) and 37.2% (n=29) were ESBL negative. The ESBL producer isolates were distributed as Proteus spp. (66.7%), Klebsiella spp. (66.7%), E. coli (52%), Pseudomonas spp. (71.42%), and Acinetobacter baumannii (60%).

Conclusion: High nosocomial infections in ICUs are caused by ESBL and MRSA, primarily due to excessive antibiotic use. These bacteria's resistance patterns provide valuable antimicrobial surveillance data, enabling restrictions on β-lactams. Combating antibiotic resistance requires surveillance, prudent antibiotic use, infection control, new antibiotic development, and phage therapy.

Keywords: Resistant strains of bacteria, Role, Hospital acquired infection, MRSA.

INTRODUCTION

Hospital acquired infection is defined as an infection acquired in hospital by a case who has been admitted for a reason other than that infection. Urinary tract infections, surgical wounds, and the lower respiratory tract are the most common types of nosocomial infections. These infections are most frequently observed in orthopedic and surgical wards, as well as in intensive care units. In non-hospitalized cases, the organisms causing majority of hospital-acquired infections can't induce illnesses or can induce a milder form of illness. This involves coagulase-negative Staphylococci, Staphylococcus aureus, Enterococci, and Enterobacteria. Factors that elevate the susceptibility of a case to nosocomial infections involve underlying disease, therapeutic and diagnostic interventions, reduced immunocompromised cases, and young or elderly age [(1)].

Drug-resistant organisms frequently induce nosocomial infections. Resistance development can be promoted by the misuse of antimicrobials for prophylaxis or management. Multidrug-resistant strains are generated via the exchange of genetic resistance elements and antimicrobial-driven selection. The resistant strains survive, while antimicrobial-sensitive microorganisms that are part of the endogenous flora are suppressed. Numerous staphylococci, pneumococci, enterococci, and tuberculosis strains are presently resistant to the majority or all antimicrobials that were previously effective [(2)].

Methicillin-resistant Staphylococcus aureus (MRSA) are cocci that have developed resistance to β-lactam antibiotics, such as ampicillin, penicillin, methicillin, amoxicillin, cephal osporins, and monobactams. MRSA have been initially identified in 1961 and has since became a significant nosocomial pathogen on a global scale. MRSA bacteraemia accounts for 40% of S. aureus bacteraemia in the United Kingdom [(3)].

This study aimed to discuss bacteriological characters, genetic structure, different methods for detection either phenotypically or genotypically, and risk factors for mortality of methicillin resistant staphylococci and extended-spectrum β-lactamase producing organisms for evaluating and limiting its emergency in hospital acquired infections.

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PATIENTS AND METHODS

This investigation has been performed on 100 cases suffering from infection, which wasn't present at the admission time to Al-Ahrar Teaching Hospital and not manifested in the first 48 hours of hospitalization i.e., nosocomial infection.

MATERIALS

Media used for routine culture: Blood agar and blood culture bottles (Oxoid cop., England), nutrient agar (Oxoid cop., England), MacConkey's medium (Oxoid cop., England), Muller-Hinton agar (Oxoid) and chromogenic media for staph aureus (Oxoid cop. England). **Reagents for Staph. aureus identification:** Mannitol-salt agar (Oxoid), hydrogen peroxide solution (H_2O_2 3%). bacitracin disks 0.04 units (Difco, USA), Fresh human plasma for coagulase test, Avipath-Staph (Omega Diagnostics, UK): a rapid latex agglutination test for the differentiation of S. aureus which produces clumping factor and/or protein A form those species of Staph., which do not and chromogenic media for staph aureus (Oxoid cop., England).

Materials used for Antibiotic susceptibility tests by disc diffusion method: Mueller Hinton agar: (Hamedia comp., India), 0.5 McFarland turbidity tube, a ruler to measure the zone of inhibition and antibiotic discs (Oxoid cop., England)**:** 1 ug oxacillin disk (Oxoid)**,** ceftazidime 30 mg, cefotaxime 30 mg, ceftazidime-clavulanate disc (20 and 10 mg respectively), and cefotaxime-clavulanate disc (20 and 10 mg respectively).

Reagents and equipment used in LightCycler PCR: DNA extraction: using MagNA Pure Compact Nucleic Acid Isolation Kit I, **DNA amplification** utilizing LightCycler-DNA Master SYBR Green I kit, which is a ready-to-use reaction mix for PCR and **detection of the amplified products using melting curves.**

Methods

All patients were subjected to the following:

Patient identification: Patients were categorized based on age, gender, underlying disease, radiation exposure, preoperative regimen, antibiotic intake, and hospitalization length. General condition was assessed through hemoglobin level, blood pressure, body weight, and random blood glucose levels. Possible sources of infection were investigated using specimens from healthcare workers, nurses, and hospital buildings. During hospitalization, patients were observed for clinical manifestations suspecting infection and diagnosed based on at least one positive culture. Samples were collected from pustulated wounds, urine, stool, sputum, and endotracheal aspiration samples. A threshold concentration of 109 CFU/ml sputum defines urinary tract infection.

Laboratory examination and identification of organisms:

Identification of the positive specimens was based on: Morphological and antibiotic susceptibility tests.

Identification of extended spectrum beta-lactamase Enterobacteriaceae

The colonies grown on MacConkey's medium, which is a selective media for Gram negative bacilli, undergo the following: Beta lactamase production was identified by using Oxoid sticks, which contains nitrocefin (chromogenic cephalosporins), which change from yellow to red or pink when beta lactam ring is hydrolyzed by beta lactamase. The change of colour occur after 15 min to 1 hours, **resistance to both ceftazidime (CAZ) and cefotaxime (CTX).**

Detection of ESBL MIC using HiComb E-test strips: The test detects the reduction in the MIC of ESBLs producing organisms to ceftazidime in the presence of clavulanic acid. The strip is impregnated with ceftazidime alone and both ceftazidime and clavulanate **(4)**. Muller Hinton agar was inoculated with a 0.5 McFarland turbidity of each isolate, dried, and applied to each plate **(5)**. The MIC is defined as the intersection of the inhibition ellipse and the of the E-test strip edge. The presence of ESBLs is indicated by a ceftazidime MIC/ceftazidime clavulanic acid MIC ratio of eight or higher (positive test) **(5). Double disc synergy method:** We used ceftazidime 30 mg, cefotaxime 30 mg, and ceftazidime and cefotaxime-clavulanate discs to test for ESBL. Muller Hinton agar plates were inoculated with a 0.05 McFarland turbidity suspension of each isolate. The antibiotic discs were placed 25 mm apart and incubated for 24 hours at 37ºC **(4)**. The sensitivity to these discs was measured by measuring the inhibition zone around the disc, translating into susceptible, intermediate susceptible, or resistant **(6).**

BBL crystal: The BBL CRYSTAL GN ID System necessitates a Gram stain and included the selection of colonies with identical morphologies using a sterile cotton swab. The colonies were vortexed for 10-15 seconds in a tube containing BBL CRYSTAL GN, ID Inoculum Fluid, and a recap tube. The colonies were suspended in the tube. The turbidity met the McFarland turbidity standard of 0.5. The base was placed on the bench top, gently rolled, and the inoculum fluid tube was poured into the base target area. Panels were incubated at 35-37°C for 18-24 hours. Panels were read within 30 mm of being removed from the incubator if they were incubated for 24 hours. The fluorescence intensity of a fluorescent substrate well was deemed positive if it exceeded that of the negative control well. In accordance

with the row in which the test is located, each positive test result (excluding 4A) was assigned a value of 4, 2, or 1, while a negative result was assigned a value of 0 (zero).

Identification of Staphylococcal isolates

Isolation of the organism: Staphylococcal isolates from different samples were subcultured on mannitol-salt agar (Oxoid). The medium's selective nature is attributed to the salt content (7.5% NaCl), which inhibits majority of organisms with the exception of staphylococci.

Gram stain: Only Gram-positive cocci in clusters were further examined by the following tests: **Catalase test, bacitracin susceptibility, tube coagulase test and latex agglutination test (Avipath-Staph**):

Phenotypic detection of methicillin resistance among staphylococcal isolates:

Chromogenic identification of S. aureus involves a combination of mannitol 1% and phenol red, with color changes indicating MRSA. Oxacillin resistance has been tested utilizing the oxacillin disc diffusion technique. PCR was used to amplify target DNA sequences. MagNA Pure Compact Nucleic Acid Isolation Kit I purified genomic DNA and total nucleic acids from blood, blood cells, and serum. MagNA Pure Magnetic Glass Particle Technology lysed samples with Proteinase K and a chaotropic salt-containing lysis buffer. The LightCycler-DNA Master SYBR Green I was a ready-to-use PCR reaction mix with SYBR Green I dye for detection. Continuous monitoring allowed visualization of reaction progress in temperature, time, and fluorescence.

Ethical considerations

The data that were collected from participants were confidential. The research participants weren't identified by name in any publication or report that addressed this research. The nature and goal of the research, as well as the risk-benefit evaluation, have been explained to the participants prior to their admission to this study. Informed consent has been obtained from each participant. Approval of Ethics committees of General Organization for Teaching Hospitals and Institutes, Al-Ahrar Teaching Hospital was obtained. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis

The IBM Statistical Package for the Social Sciences (SPSS) software version 22.0 has been utilized to statistically analyze and present all of the data that was gathered in tables. Frequency and percentages were used to present categorical data, which have been compared utilizing chi-square tests. Statistical significance was defined as a p value that was less than 0.05.

RESULTS

This table showed that although the infection was more prevalent over age of 40 years, yet there was statistically insignificant relation between development of nosocomial infections and age groups. The study included 100 patients, 48 males and 52 females who developed nosocomial infections (Table 1).

Table (1): Distribution of nosocomial infections by age and sex of the studied patients.

Age (years)	Sex					
	Males		Females		Total	
	No	%	No	%	No	%
2-20	7	36.8	12	63.2	19	100.0
21-40	6	66.6	3	33.4	9	100.0
41-50	13	43.3	17	56.7	30	100.0
51-70	18	56.3	14	43.7	32	100.0
70 +	4	40.0	6	60.0	10	100.0
Total	48	48%	52	52.0%	100	100.0

Chi-square = 3.6 P value = 0.464 Statistically insignificant

Proteus species, MRSA, and Acinetobacter baumannii were represented as isolates from others (eye, peritoneal, vaginal). The highest were Proteus. 4 samples were collected and processed for bacteriological study (Table 2).

Table (2): Distribution of different isolated organisms from other NIs (as eye, vaginal infections).

Isolated organisms	No	%
Proteus	2	(50%)
MRSA	1	(25%)
Acinetobacter baumannii	1	(25%)
Total	4	(100%)

MRSA: methicillin-resistant Staphylococcus aureus

The frequency distribution of microorganism by different sites of infection in the body of patients suffering from nosocomial infections is shown in table 3. The E. coli represented the majority % of organisms. (Table 3).

Table (3): Distribution of different isolated organism from different sites in the body of the patients suffering from nosocomial infections

The organism	Sputum	Urine Samples	Pus samples	Blood	Other samples	Total %
E. coli	**11 (44%)**	**8 (32%)**	**3 (12%)**	**3 (12%)**	**0 (0.0%)**	**25 (100.0)**
MRSA	**8 (36.36%)**	**3 (13.63%)**	**9 (40.9%)**	**1 (4.54%)**	**1 (4.54%)**	**22 (100.0)**
Pseudomonas spp.	**14 (66.66%)**	**4 (19.04%)**	**3 (14.28%)**	**0 (0.0%)**	**0 (0.0%)**	**21 (100.0)**
Klebsiella spp.	**10 (55.55%)**	**6 (33.33%)**	**0 (0.0%)**	**2 (11.11%)**	**0 (0.0%)**	**18 (100.0)**
Proteus spp.	**2 (2.22%)**	**2 (22.22%)**	**2 (22.22%)**	**1 (11.11%)**	**2 (22.22%)**	**9 (100.0)**
A. baumannii	**2 (40.0%)**	**0 (0.0%)**	**1 (20.0%)**	**1 (20.0%)**	**1 (20.0%)**	**5 (100.0)**
Total	**47 isolates**	**23 isolates**	**18 isolates**	**8 isolates**	**4 isolates**	**100 isolates**

E. coli: Escherichia coli

This table presents the interpretation of initial screening test results for suspected extended-spectrum beta-lactamase (ESBL) production. The zone diameter of antimicrobial disks is used to assess bacterial resistance. For ceftazidime (CAZ) 30 µg, a zone diameter of ≤22 mm indicated resistance, while for cefotaxime (CTX) 30 µg, a zone diameter of ≤27 mm suggested resistance. These results are crucial in identifying bacteria that may produce ESBLs, which can hydrolyze and inactivate a broad range of beta-lactam antibiotics, guiding appropriate treatment options (Table 4).

Table (4): Reading of initial screening test results that suspecting extended spectrum beta lactamase production

Antimicrobial Disk Concentration	Zone Diameter
Ceftazidime (CAZ) 30µg	≤22mm: Resistant
Cefotaxime (CTX) 30µg	≤27mm: Resistant

The table outlines the interpretation of minimum inhibitory concentration (MIC) values obtained using HiComb MIC strips. It categorized bacterial resistance to antimicrobial agents as follows: a MIC value of ≤8 indicated sensitivity to the antimicrobial agent, values between 9 and 31 represented intermediate resistance, and a MIC value of ≥32 signified resistance. This classification helped in determining the effectiveness of the antimicrobial agent against specific bacterial strains (Table 5).

Table (5): Reading of MIC values by HiComb MIC strips

Sensitive≤8	Intermediate 9 – 31	Resistant ≥32

From the 78 potentially ESBL producer isolates by the screening tests, confirmed ESBL has been detected in 62.8% (n=49). The ESBL producer isolates were distributed as Pseudomonas spp. (71.42%), Proteus spp. (66.7%), Klebsiella spp. (66.7%), Acinetobacter baumannii (60%), and E. coli (52%) (Table 6).

Table (6): Frequency of ESBL positive organisms in all samples by confirmatory methods

	By confirmatory method				
Organisms	**ESBL positive**		**ESBL negative**		**Total**
	No	%	No	%	No and %
E. coli spp.	13	52.0	12	48.0	(100.0)
Pseudomonas spp.	15	71.42	6	28.58	(100.0)
Klebsiella spp.	12	66.7	6	33.3	(100.0)
Proteus spp.		6.7		3.3	9 (100.0)
Acinetobacter baumannii		0.0		0.0	5 (100.0)
Total	49	62.8 %	29	37.2 %	78 (100.0)

ESBL: Extended Spectrum Beta-Lactamase

For S. aureus mecA PCR, 75% of the isolates were tested positive for the mecA gene, indicating methicillin resistance. In the case of CoNS mecA PCR, 60% of the isolates were tested positive for the mecA gene. For Confirmed ESBL: β-lactamase (bla) gene PCR, 77.5% of isolates were positive for the ESBL gene. These results highlight the prevalence of resistance mechanisms such as mecA and bla genes in the studied bacterial populations, providing insight into antimicrobial resistance patterns (Table 7).

Table (7): Prevalence of the resistant organisms by PCR:

	Positive	Negative	Total
S. aureus mecA PCR	9 (75.0%)	3 (25.0%)	12 (100.0%)
CoNS mecA PCR	6 (60.0%)	4 (40.0%)	10 (100.0%)
Confirmed ESBL: β-lactamase (bla) gene PCR	38 (77.5%)	11 (22.5%)	49 (100.0%)

CoNS= Coagulase negative staphylococcus aureus.
ESBL= Extended spectrum beta lactamase Enterobacteriaceae

Sensitivity of confirmatory tests in comparison to PCR to detect ESBL was 61.25% while specificity was 67.3%. Sensitivity of ordinary methods in comparison to PCR to detect MRSA was 59.5% while specificity was 63.6% (Table 8).

Table (8): Prevalence of bla positive and negative of ESBLs isolates by PCR:

Organisms	bla positive	bla negative	Total
Klebsiella spp.	12 (100%)	0 (0.0%)	12 (100.0%)
E. coli	11 (84.6%)	2 (15.4%)	13 (100.0%)
Pseudomonas	10 (66.7%)	5 (33.3 %)	15 (100.0%)
Proteus	3 (50.0%)	3 (50%)	6 (100.0%)
Acinetobacter baumannii	2 (66.7%)	1 (33.4%)	3 (100.0%)
Total	38 (77.5%)	11 (22.5%)	49 (100.0%)

Chi-square = 7.69 P value = 0.103, Statistically insignificant

DISCUSSION

In the present study, 52% of patients with NIs were females, this agrees with many studies that show higher incidence in females to NIs than males. **Ohkawa *et al.*** [7] and **Harding *et al.*** [8] recorded female's incidence of 10 (17.5%), 119 (100%) respectively. This may be due to many predisposing factors; their anatomical short urethra which increased incidence of UTIs and obesity, which may predispose to RTIs [3].

In this study 43.7% of females infected with NIs were of age group between 51-70 years. In similar study by **Gharib *et al.*,** [9], incidence of NIs was 13% among females of age group (22-44 years), 13.9% in age group of (44-66 years) and 18.5% in age group of (66-88 years). This also could be due to decrease in sex hormones, stress, and decrease immunity accompanying with age [10].

Regarding the frequency distribution of microorganism by different sites of infection in the body of patients suffering from nosocomial infections. The E. coli represented the majority % of organisms . In the present study 78% of nosocomial pathogens were GNB followed aureus (MRSA and CoNS).

Similar outcomes have been also acquired by **Hanberger *et al.*** [14] who reported GNB incidence of 60%.

In developed countries at least 5% of patients admitted to hospitals develop NIs by GNB.

Detection of ESBL production is determined by different methods. In our study the recorded ESBL production by E-test and resistance to both CAZ and CTX was 78 isolates. These potential ESBL producers are confirmed to produce ESBL by double disc diffusion method. Only 49 isolates (62.82%) were confirmed as ESBL producers. Other study done by **Cormican *et al.*** [5] recorded higher results using double disc diffusion method 89%.

In the current investigation, overall ESBLs occurrence in our isolates was 62.82%. It is significantly higher than the rate of increase in Europe, which has increased from 15.79% in 2002 to 17.8% in 2005 [15], as well as in Asia [13]. The global prevalence of ESBL-producing isolates among different Enterobacteriaceae species ranges from less than 1% to 74% [16].

The current results highlight the prevalence of resistance mechanisms such as mecA and bla genes in the studied bacterial populations, providing insight into antimicrobial resistance patterns. compared to PCR for mecA. Many studies used PCR for mecA as the gold standard for diagnosis of methicillin resistance [17]. However, the high cost of PCR necessitated the search for another reliable technique.

ESBL production is more definitively defined by tests that rely on the detection of ESBL by PCR. Two types of discordance have been observed in this investigation when the outcomes of the combined disk test have been compared to those of the PCR. Eleven isolates have been found to be positive for ESBL in the phenotypic test but negative in the PCR. Depending on the inhibition of the enzyme by the clavulanic acid, these outcomes suggest a potential detection failure in the test.

Pitout *et al.* [15] stated that the combined disk technique may fail to detect ESBLs due to hyper-production of SHV-1 or SHV-11. Nevertheless, this type of resistance detection was even more effective when PCR has been utilized. This outcome confirms the known limitations of phenotypic techniques in a scenario of elevating microbial resistance to antibiotics, thereby reinforcing the necessity of molecular techniques, like PCR, for ESBL-producing microorganism's identification.

CONCLUSION

Nosocomial infections in ICUs are high due to the prevalence of Extended-spectrum beta-lactamases (ESBLs) and MRSA. The excessive use of antibiotics in hospitals contributes to these infections. The frequency and resistance patterns of these bacteria provide valuable antimicrobial surveillance information, enabling restrictions on β-lactams. Antibiotic-resistant bacteria increase morbidity, mortality, and hospital stay duration. Combating antibiotic resistance requires surveillance, prudent antibiotic use, effective infection control, new antibiotic development, and phage therapy.

DECLARATIONS

- **Consent for publication:** I certify that each author has granted permission for the work to be submitted.
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- **Availability of data and material:** Available.
- **Conflicts of interest:** None.
- **Competing interests:** None.

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