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Measurement of Some Inflammatory Biomarkers and Genotyping of Gram Negative Bacteria Isolated from Acute Leukemia Patients

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Abstract

This study detected the prevalence of gram-negative bacteria (GNB) among Iraqi acute leukemia patients and measured their serum levels for C- reactive protein (CRP), procalcitonin (PCT) and lipopolysaccharide- binding protein (LBP). Besides, random amplification of polymorphic DNA (RAPD) typing was utilized to investigate the genetic relationship among GNB isolates. Out of 458 clinical samples collected from 260 acute leukemia patients, 70 (15%) isolates of GNB were diagnosed as *Klebsiella pneumoniae* 23 (33%), *Escherichia coli* 21 (30%), *Pseudomonas aeruginosa* 18 (26%) and *Acinetobacter baumannii* 8 (11%). These isolates were collected from urine (39 (57%)), blood (23 (32%)) and swabs (8 (11%)). Furthermore, GNB infections were higher among patients with acute myeloblastic leukemia (AML) (40 (57%)) than those with acute lymphoblastic leukemia (ALL) (30 (43%)). The concentrations of CRP, PCT and LBP recorded higher levels among men, women and children groups than control groups. Most of GNB isolates were highly resistant to ticarcillin, piperacillin, ceftazidime and cefepime, followed by gentamicin, tobramycin and ciprofloxacin. Imipenem and meropenem were more effective against GNB isolates, except for *A. baumannii*. Multidrug resistant (MDR) pattern was noticed among all isolates of *K. pneumoniae* (23 (100%)) and *A. baumannii* (8 (100%)), whereas 18 (85%) and 12 (66%) of *E. coli* and *P. aeruginosa* respectively showed MDR pattern. RAPD typing yielded different genotypic patterns among GNB isolates exceeded 14, 12, 11 and 9 in *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *A. baumannii* respectively. The increased serum levels of CRP, PCT and LBP indicated the possible vital role of these markers in predicting the severe infections of GNB in acute leukemic population. The genetic diversity among GNB isolates surveyed by RAPD typing requires the continuous monitoring of the prevalence of pathogenic GNB associated with specific genotypic pattern.

Keywords: Leukemia, Gram negative bacteria, RAPD, Procalcitonin, Lipopolysaccharide- binding protein

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قياس بعض المؤشرات الحيوية الالتهابية و التنميط الجيني للبكتيريا سالبة الكرام المعزولة من مرضى ابيضاض الدم الحاد

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الخلاصة:

تحرت هذه الدراسة عن انتشار البكتيريا سالبة الكرام في المرضى العراقيين المصابين بمرض ابيضاض الدم الحاد مع قياس مستويات البروتين التفاعلي سي CRP و البروكالسيتونين PCT و البروتين المرتبط باللبيد متعدد السكريدLBP في المصل. كما تم التحري عن القرابة الوراثية بين العزلات البكتيرية بأستخدام تقنية RAPD. جمعت 458 عينة سريرية من 260 مريض و بعد التشخيص تم الحصول على 70 (15%) عزلة من البكتيريا سالبة الكرام شملتالكلبسيلة الرئوية23 (33%) والاشيريكية القولونية21 (30%) و الزائفة الزنجارية 18 (26%) والراكدة البومانية 8 (11%). جمعت العزلات من الادرار 39 (57%)، الدم 23 (32%) و المسحات 8 (11%). اظهرت النتائج ان العزلات البكتيرية عزلت بنسبة اعلى من مرضى ابيضاض الدم النخاعي الحاد 40 (57%) مما هو عليه في مرضى ابيضاض الدم الليمفاوي الحاد 30 (43%). اظهرت تراكيز CRP و PCT و LBP مستويات عالية في مجموعات المرضى الرجال و النساء و الاطفال مقارنة مع المجموعات الضابطة. اظهرت معظم العزلات مقاومة عالية تجاه مضادات ticarcillin، piperacillin، ceftazidime و cefepime تليها المضادات gentamicin، tobramicin و ciprofloxacin. ان مضادي imipenem و meropenem كانا اكثر فاعلية تجاه العزلات البكتيرية بإستثناء عزلات الجنس الراكدة البومانية. تم تشخيص نمط المقاومة المتعددة للمضادات الحيوية في جميع عزلات بكتيريا الكلبسيلة الرئوية23 (100%) و الراكدة البومانية 8 (100%) بينما تم تشخيص هذا النمط في 18 (85%) و 12 (66%) من عزلات بكتيريا الاشيريكية القولونية والزائفة الزنجارية على التتالي. انتجت تقنية RAPD انماط وراثية مختلفة ضمن العزلات البكتيرية بلغت 14 و 12 و 11 و 9 في بكتيريا الزائفة الزنجارية و الاشيريكية القولونية و الكلبسيلة الرئوية و الراكدة البومانيةعلى التتالي. ان ارتفاع مستويات CRP و PCT و LBP في المصل يشير الى الدور الحيوي المحتمل لهذه المؤشرات الحيوية في التنبؤ بالاصابات الخطيرة التي تسببها البكتيريا سالبة الكرام في مرضى ابيضاض الدم الحاد. ان التنوع الوراثي الذي تم التحري عنه بتقنية RAPD يتطلب مراقبة مستمرة لانتشار عزلات البكتيريا سالبة الكرام الممرضة و خصوصا تلك المرتبطة بنمط وراثي معين.

Introduction

Acute leukemia is a complex disease characterized by a malignant transformation of hematopoietic stem cells which arise from the bone marrow [1]. This abnormal leukemic population of cells suppresses the production of normal blood cellular components, especially leukocytes. Broadly, acute leukemia can be divided into two main subtypes, namely acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML) as these are most likely to be encountered by physicians [2].

Leukemic patients are usually immunocompromised due to clinical treatment by chemotherapy, radiotherapy, impairment function of leukocytes and use of immunosuppression drugs [3]. This becomes even more complicated when the patients develop risk infectious disorders like sepsis, septic shock and febrile neutropenia. The patients

are at high risk if they get infected with opportunistic gram-negative bacteria (GNB). Hence, early identification of bacterial infections is crucial to reduce morbidity and mortality [4].

The administration of broad-spectrum antibiotics or combination of antimicrobials against bacterial infections can significantly lead to the incidence and dissemination of multidrug resistant (MDR) bacterial strains, in addition to adverse reactions of antibiotics and increasing cost of health care [5].

The evaluation of inflammatory biomarkers might serve as a quick and indirect evidence of bacterial infections and may also help to optimize antimicrobial therapy in this high-risk population [4]. This serves to avoid the delay in obtaining the results from the culture of the pathogenic bacteria and improve the outcome of patients [5].

C- reactive protein (CRP) and procalcitonin (PCT) are the most widely assayed biomarkers among leukemic patients [6]. CRP is an acute phase protein that is mainly released by the liver that reacheshigh concentrations through inflammatory response triggered by bacterial infections [6].

Procalcitonin is the precursor of the hormone calcitonin that is secreted by the C- cells of the thyroid gland [7]. PCT is superior to CRP for predicting bacterial antigens as its levels raise significantly faster than of CRP at the early stage of infection, reflecting the severity of infection [6].

Lipopolysaccharide- binding protein (LBP) is another inflammatory biomarker that is measured in the early detection of bacterial infection, especially in critically ill neonates and children who suffer from leukemia [8]. It is mainly produced by the liver as an acute phase protein and binds with high affinity with the lipopolysaccharide (LPS), the antigenic components of gram-negative bacterial cell wall [8]. LBP acts by transporting LPS to the CD14 receptors on the surface of monocytes and macrophages, stimulating the production of inflammatory cytokines [8].

It is an important to understand the distribution and relatedness of pathogenic GNB isolates in order to track the epidemiological routes of the most common resident MDR GNB isolates expanded in the hospital environment to reduce their risk nosocomial infections. Molecular typing methods are now the most accurate methods to achieve this purpose [9]. The random amplification of polymorphic DNA (RAPD) is among the common molecular methods used in bacterial typing as it is simple, rapid, inexpensive and easy in use [9].

This study aimed to measure the serum levels of CRP, PCT and LBP in acute leukemia patients infected with GNB infections and determine the genetic relatedness among these isolated GNB by RAPD typing.

Material and Method

Sampling and Criteria

This study included 260 patients, aged from 1 to 65 years with acute leukemia, who were admitted to hematology center at Baghdad Teaching Hospital, Children Welfare Teaching Hospital and Central Teaching Hospital of Pediatric between January 2021 and December 2021. Of the patients, 156 (60%) were females aging between 1 to 63 years and 104 (40%) males between the ages of 1 and 65 years. Among these patients, 132 (51%) and 128 (49%) were diagnosed as AML and ALL respectively by specialist hematologists.

Inclusion criteria involved type of acute leukemia, gender and age obtained from the medical charts of patients. Exclusion criteria had patients who developed viral and fungal infections, patients received antibiotics before blood culture was made and those unwilling to participate in this study. The patients were divided according to gender and age into three groups which included men's group (18-65 years), women's group (18-63 years) and children group (1-14 years). The control group included healthy volunteers with same genders and ages of patients groups.
Blood and urine samples were collected each from 198 patients (198 (43%) blood and 198 (43%) urine samples), whereas swabs were collected from 62 (14%) patients with skin injuries and abscesses.

This study was approved by the Medical Ethical Committee of Baghdad Medical City following the approval 300294. Written consent was obtained from the adult patients and children's parents to share the results of this study.

Laboratory Diagnosis

Blood samples were drawn in both blood culture bottles containing brain heart infusion broth (AFCO, Jordan) and gel tube (Biozek, Holland) for each patient. Broth cultures were incubated (Memmert, Germany) at 37℃ for 24 h and subculture was performed twice a day for the first two days and then daily for one week on MacConkey agar, blood agar, and chocolate agar media (NEOGEN, UK) [10]. Besides, urine and swab samples were immediately cultured on the aforementioned agar media and incubated under the same conditions [10].

The pure bacterial colonies obtained from positive cultures were firstly identified morphologically by Gram stain (AFCO, Jordan) [10]. Only GNB were selected and were further identified by conventional biochemical tests that included oxidase, catalase and indole tests and confirmed by Vitek 2 Compact System (Biomerieux, France) using ID GN cards [10, 11].

The gel tubes were centrifuged (Fisher Scientific, USA) at 2000 rpm for 10 min to yield sera stored (Sysmix, Japan) at -80℃ until using [10]. CRP, PCT and LBP were measured using enzyme linked immunosorbent assay (ELISA) kits purchased from CUSABIO (USA). The optical density of the serum and standard samples was measured at 450 nm using the microtiter plate reader (Human Reader HS, Germany) [10].

Antibacterial Susceptibility Test (AST)

The susceptibility for nine antibiotics from different classes was determined for all identified GNB isolates by detecting the minimum inhibitory concentrations (MICs) using Vitek 2 Compact System. These antibiotics comprised of ticarcillin (16, 32, 64 μg/mL), piperacillin (4, 16, 32, 64 μg/mL), ceftazidime (1, 2, 8, 32 μg/mL), cefepime (2, 8, 16, 32 μg/mL), imipenem (1, 2, 6, 12 μg/mL), meropenem (0.5, 2, 6, 12 μg/mL), gentamicin (4, 16, 32 μg/mL), tobramycin (8, 16, 64 μg/mL) and ciprofloxacin (0.5, 2, 4 μg/mL). The bacterial inoculum was prepared for all isolates and the turbidity was adjusted to an equivalent of 0.5 McFarland standards [11]. From each inoculum, 145 μl was transferred into 3 mL normal saline in a second tube. This suspension was used to charge the wells of AST cards and then incubated and read [11]. The isolates were considered as MDR according to its resistance to at least three antibiotics from different categories [11].

Genomic DNA extraction and RAPD Typing

Genomic DNA of all GNB isolates was extracted from overnight broth culture using ABIO pure™ total DNA kit (USA), according to the instructions of manufacturing company and stored at -70℃. The concentrations and purity of the extracted DNA were detected by nanodrop (Biogroup, UK) at a wave-lengths 260 and 280 nm [12].

Random amplification of polymorphic DNA polymerases chain reaction (RAPD PCR) analysis was carried out using the primer 272 (5′-AGCGGGCCAA-3′) supplied by Alpha DNA (Canada) (13). PCR was performed at a reaction volume of 20 µl consisting of 10 µl GoTaq® Master Mix (Promega, USA), 2 µl RAPD 272 primer, 2 µl template DNA, and 6 µl nuclease free water (Promega, USA). The amplification was conducted by Thermal Cycler (Fisher Scientific, USA) under the following conditions: initial denaturation at 94℃ for 2 min, 35 cycles of denaturation at 94˚C for 30 s, annealing at 35℃ for 30 s, extension at 72℃ for 2 min and a final extension at 72℃ for 10 min [13].

The amplified RAPD PCR products were then separated by electrophoresis (Thermo, USA) on 2% agarose gel stained with 0.5 µg/mL ethidium bromide (Promega, USA). The run was conducted in 1X TAE buffer (Promega, USA) at 50 V for 100 min and visualized by UV Transilluminator (Major Science, Taiwan). DNA ladder (250-10000 bp) from Promega (USA) was used to evaluate the molecular weight of the separated bands [12].

Dendrogram construction was generated by the unweighted pair grouping method with arithmetic averages (UPGMA) [13].

Statistical Analysis

The non parameters results were described as number and percentage. The parameters were presented as Mean±S.E. using SPSS program (version 25) using one way ANOVA test by obtaining least significant differences (LSD). The differences at $P \leq 0.05$ were considered significant.

Results and Discussion

The laboratory analysis for 458 clinical samples included 198 (43%) blood, 198 (43%) urine and 62 (14%) swab samples yielded 70 (15%) isolates that initially identified as GNB which comprised *Klebsiella pneumoniae* 23 (33%), *Escherichia coli* 21 (30%), *Pseudomonas aeruginosa* 18 (26%) and *Acinetobacter baumannii* 8 (11%). Most of the identified GNB isolates were collected from urine 39 (57%), followed by blood 23 (32%) and swabs 8 (11%) (Table 1). The infections of GNB were diagnosed in 40 (57%) and 30 (43%) of patients with underlying diseases of AML and ALL respectively. Moreover, the identified GNB were determined among 42 (60%) and 28 (40%) of females and males respectively and among 39 (56%), 24 (34%) and 7 (10%) of women, men and children groups respectively (Table 2).

Table 1: Distribution of clinical samples.

Sample Source	Pathogen n (%)	Total n (%)
Urine	*K. pneumoniae* 13 (19) *E. coli* 13 (19) *P. aeruginosa* 6 (9) *A. baumannii*7 (10)	39 (57)
Blood	*K. pneumoniae* 8 (11) *E. coli* 8 (11) *P. aeruginosa* 6 (9) *A. baumannii*1 (1)	23 (32)
Swabs	*K. pneumoniae* 2 (32) *P. aeruginosa* 6 (9)	8 (11)

Table 2: Characteristics of patients

Characteristics	Total Patients n (%)	Patients with GNB Infections n (%)
Type of leukemia		
AML	132 (51)	40 (57)
ALL	128 (49)	30 (43)
Gender		
Male	104 (40)	28 (40)
Female	156 (60)	42 (60)
Age Groups		
Men group	117 (45)	24 (34)
Women group	56 (22)	39 (56)
Children group	87 (33)	7 (10)

AML: acute myeloblastic leukemia, ALL: acute lymphoblastic leukemia.

Similar to our finding, some previous studies [14, 15, 16] also demonstrated that *K. pneumoniae* was the predominant species among their GNB isolates, followed by *E. coli*, *P. aeruginosa* and *A. baumannii*. In contrast, other studies [17, 18, 19, 20] found that *E. coli* was the most isolated species among GNB isolates, followed by *K. pneumoniae*, *P. aeruginosa* and *A. baumannii*.

Concerning serologic assay, 15 patients infected with GNB were selected each from men, women and children groups, and the same distribution was performed with the versus non infected control groups. The mean levels of CRP were 8.50±0.75, 6.24±0.76, and 6.30±0.45 ng/mL for men, women, and children groups respectively as compared to the control groups. Statistically, CRP levels showed significant differences each between patients and control groups ($P \leq 0.05$). Moreover, the differences among all patients groups were also significant ($P \leq 0.05$). Procalcitonin had mean levels that reached 323.55±35.60, 290.55±25.39 and 226.90±21.95 pg/mL, in men, women and children's groups respectively, versus the control groups. Statistically significant differences ($P \leq 0.05$) were found in men and women groups versus the control groups, whereas children group did not ($P > 0.05$). Furthermore, no significant differences were observed among all patients groups.

High CRP and PCT levels were recorded by several studies when they assayed these markers in hematological malignant patients that included acute leukemia patients infected with GNB [4, 21, 22, 23, 24]. CRP has been widely used in clinical practice, however PCT has better diagnostic accuracy for bacteremia than CRP [7]. This is due to the fact that PCT peaks at 6 hours after bacterial induction with half- life 25 - 30 hours in plasma, while CRP peaks at 36 - 50 hours with half- life 4 - 7 hours in plasma [7]. Evaluation of the increased CRP and PCT rates can greatly aid in early prediction of bacteremia in acute leukemia patients, especially those who developed risk disorders like febrile neutropenia, sepsis and septic shock [23]. Additionally, close monitoring of inflammatory markers might help hematologists to provide antibiotic regimen in treatment of patients, avoiding the dissemination of MDR clones of GNB [21].

The LBP levels were 13.27±1.27, 8.90±0.73, and 9.67±0.31 pg/mL in men, women and children patients groups respectively as compared with those in control groups. The statistical differences were observed to be significant each between patients and control groups ($P \leq 0.05$) but did not among all patient's groups ($P > 0.05$).

Early publications [25, 26, 27] found that LBP concentrations were high in children with bacterial infections compared with the infected adults, but Kitanovski *et al*. [27] found no

significant differences between patients and the control group. They found that LBP had proven superior to CRP, PCT, and interleukin-6 in predicting bacteremia and sepsis in critically ill newborns and children. This can be explained as a result of the physiological variations between LBP and other markers among heterogenous populations of children and adults [25]. However, further studies are needed to elucidate this issue. The elevated level of LBP can be attributed to the incidence of endotoxemia as a result of LPS entering into the blood circulation due to the mucosal barrier disruption [25].

Although many studies have demonstrated significantly higher statistical differences in CRP, PCT and LBP levels, still there is a difference in biomarkers values between this study and others. This can be interpreted by the difference in sample size, the study could be conducted in a single center, number of underlying groups, the quality of techniques utilized in the evaluation of biomarkers, severity of disease, infected with viral and fungal infections and the probability of receiving chemotherapeutic agents prior to admission at hospital [21, 23].

Antimicrobial susceptibility findings showed that most of GNB isolates were highly resistant to penicillin (ticarcillin and piperacillin) and third generation cephalosporins (ceftazidime and cefepime), followed by aminoglycosides (gentamicin and tobramycin) and ciprofloxacin. The carbapenems (imipenem and meropenem) were somewhat more effective against GNB isolates (Table 3).

Table 3: Antimicrobial susceptibility profile of GNB isolates.

Antibiotic	***K. pneumoniae*** **MIC ResistanceRange (µg/mL) N(%)**	***E. coli*** **MIC Resistance Range (µg/mL) N(%)**	***P. aeruginosa*** **MIC Resistance Range (µg/mL) N(%)**	***A. baumannii*** **MIC Resistance Range (µg/mL) N(%)**
Ticarcillin	≥128 23 (100)	≥128 21 (100)	≥128 12 (66)	≥128 8 (100)
Pipracillin	≥128 23 (100)	≥128 19 (90)	≥128 12 (66)	≥128 8 (100)
Ceftazidime	16-≥64 22 (95)	16-≥64 16 (76)	32-≥64 9 (50)	≥64 8 (100)
Cefepime	16-≥64 19 (82)	16-≥64 11 (52)	32-≥64 9 (50)	32-≥64 8 (100)
Imipenem	4-≥16 17 (73)	≥16 7 (33)	8-≥16 10 (55)	≥16 8 (100)
Meropenem	≥16 17 (73)	8-≥16 6 (28)	≥16 8 (44)	8-≥16 8 (100)
Gentamicin	≥16 20 (86)	≥16 5 (23)	≥16 11 (61)	≥16 6 (75)
Tobramycin	≥16 22 (95)	≥16 8 (38)	≥16 10 (55)	≥16 5 (62)
Ciprofloxacin	2-≥4 22 (95)	≥4 16 (76)	≥4 12 (66)	≥4 8 (100)

MIC: minimum inhibitory concentrations.

The antimicrobial susceptibility profile of *K. pneumoniae* revealed that all 23 (100%) isolates were resistant to ticarcillin and piperacillin. Moreover, *K. pneumoniae* isolates exhibited high resistance rates reaching 22 (95%) each for ceftazidime, tobramycin and ciprofloxacin and 20 (86%) and 19 (82%) for gentamicin and cefepime respectively. On the other hand, both imipenem and meropenem displayed lower resistance rates against *K. pneumoniae* isolates which exceeded 17 (73%) each for them.

The resistance pattern of *E. coli* showed high resistance rates both for ticarcillin 21 (100%) and piperacillin 19 (90%). Furthermore, moderate resistance rates reached 16 (76%) both for ceftazidime and ciprofloxacin, and 11 (52%) for cefepime. The reduced resistance rates were recorded 8 (38%), 7 (33%), 6 (28%), and 5 (23) for tobramycin, imipenem, meropenem and gentamicin respectively.

P. aeruginosa exhibited low resistance rates reaching 12 (66%) each for ticarcillin, piperacillin, and ciprofloxacin, 11 (61%) for gentamicin, 10 (55%) each for imipenem and tobramycin, and 9 (50%) each for ceftazidime and cefepime. Meropenem showed remarkable reduced resistance rates that reached 8 (44%).

All *A. baumannii* isolates 8 (100%) were resistant to all antimicrobials, except 6 (75%) and 5 (62%) of isolates which were resistant to gentamicin and tobramycin respectively.
Furthermore, the antibiotic resistance patterns for all studied GNB species were determined, finding that *K. pneumoniae* isolates were distributed into 8 antibiotic resistance patterns (Table 4). Most of these patterns showed resistance to ticarcillin, piperacillin, ceftazidime, tobramycin and ciprofloxacin and most isolates 16 (69.56%) were observed to be belonging to the A1 pattern. Additionally, the tested *E. coli* isolates were grouped into 8 resistance patterns (Table 5). The patterns A1, A2 and A8 were more predominant which included 14 (66.66%) isolates. Most of these patterns exhibited resistance to ticarcillin, piperacillin, ceftazidime, and ciprofloxacin. Alongside, *P. aeruginosa* isolates were arranged into 6 resistance patterns (Table 6). Of these, A1 and A6 showed more prevalent patterns, as 13 (72.22%) isolates were belonged to both patterns. The isolates among these patterns were more resistant to ticarcillin, piperacillin, and ciprofloxacin. The isolates of *A. baumannii* were classed into 3 patterns, since A1 and A2 showed more accidental patterns which contained 7 (87.5%) isolates (Table 7). The isolates among both patterns were resistant to most or all tested isolates.

Table 4: Antibiotic resistance patterns of *Klebsiella pneumoniae* isolates

Resistance Pattern	Isolates n (%)	Antibiotics
A1	16 (69.56)	TIC, PIP, CAZ, FEP, IMP, MEM, GM, TM, CIP
A2	1 (4.34)	TIC, PIP, CAZ, FEP, IMP, MEM, GM, TM
A3	1 (4.34)	TIC, PIP, CAZ, FEP, IMP, MEM, TM, CIP
A4	1 (4.34)	TIC, PIP, CAZ, FEP, IMP, GM, TM, CIP
A5	1 (4.34)	TIC, PIP, CAZ, GM, TM, CIP
A6	1 (4.34)	TIC, PIP, CAZ, TM, CIP
A7	1 (4.34)	TIC, PIP, GM, TM, CIP
A8	1 (4.34)	TIC, PIP, CIP

A: Antibiogram, TIC: Ticarcillin, PIP: Piperacillin, CAZ: Ceftazidime, FEP: Cefepime, IMP: Imipenem, MEM: Meropenem, GM: Gentamicin, TM: Tobramycin, CIP: Ciprofloxacin.

Table 5: Antibiotic resistance patterns of *Escherichia coli* isolates.

Resistance Pattern	Isolates n (%)	Antibiotics
A1	6 (28.57)	TIC, PIP, CAZ, FEP, IMP, MEM, GM, TM, CIP
A2	4 (19.04)	TIC, PIP, CAZ, FEP, IMP, MEM, CIP
A3	2 (9.52)	TIC, PIP, CAZ, FEP, TM, CIP
A4	2 (9.52)	TIC, PIP, CAZ, TM, CIP
A5	1 (4.76)	TIC, PIP, CAZ, CIP
A6	1 (4.76)	TIC, PIP, CAZ
A7	1 (4.76)	TIC, PIP, CIP
A8	4 (19.04)	TIC, PIP

A: Antibiogram, TIC: Ticarcillin, PIP: Piperacillin, CAZ: Ceftazidime, FEP: Cefepime, IMP: Imipenem, MEM: Meropenem, GM: Gentamicin, TM: Tobramycin, CIP: Ciprofloxacin.

Table 6: Antibiotic resistance patterns of *Pseudomonas aeruginosa* isolates

Resistance Pattern	Isolates n (%)	Antibiotics
A1	7 (38.88)	TIC, PIP, CAZ, FEP, IMP, MEM, GM, TM, CIP
A2	2 (11.11)	TIC, PIP, CAZ, FEP, IMP, GM, TM, CIP
A3	1 (5.55)	TIC, PIP, IMP, MEM, GM, TM, CIP
A4	1 (5.55)	TIC, PIP, GM, GM, CIP
A5	1 (5.55)	TIC, PIP, CIP
A6	6 (33.33)	Sensitive for all antibiotics

A: Antibiogram, TIC: Ticarcillin, PIP: Piperacillin, CAZ: Ceftazidime, FEP: Cefepime, IMP: Imipenem, MEM: Meropenem, GM: Gentamicin, TM: Tobramycin, CIP: Ciprofloxacin.

Table 7: Antibiotic resistance patterns of *Acinetobacter baumannii* isolates

Resistance Pattern	Isolates n (%)	Antibiotics
A1	4 (50)	TIC, PIP, CAZ, FEP, IMP, MEM, GM, TM, CIP
A2	3 (37.5)	TIC, PIP, CAZ, FEP, IMP, MEM, TM, CIP
A3	1 (12.5)	TIC, PIP, CAZ, FEP, IMP, MEM, CIP

A: Antibiogram, TIC: Ticarcillin, PIP: Piperacillin, CAZ: Ceftazidime, FEP: Cefepime, IMP: Imipenem, MEM: Meropenem, GM: Gentamicin, TM: Tobramycin, CIP: Ciprofloxacin.

The study carried out by Kamel *et al*. [17] found that *E. coli*, *K. pneumoniae* and *A. baumannii* showed high resistance for ceftazidime and cefepime, except *P. aeruginosa* which showed lower resistance for these agents. Imipenem and meropenem were more effective against their isolates. Also, they found gentamicin and ciprofloxacin had better antimicrobial action against *P. aeruginosa*. The same results were obtained by Al-Lami *et al*. [28]. Moreover, Elbadawi *et al*. [15] gained high resistance values for ampicillin, ceftazidime, ciprofloxacin, and gentamicin among their GNB isolates which included *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii*. They recorded lower resistance for imipenem and meropenem. The study of Haji *et al*. [20] revealed that all their GNB isolates (*E. coli*, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii*) were highly resistant to piperacillin, cefepime,

ceftazidime, except *P. aeruginosa* which showed lower resistance for ceftazidime. In addition, their isolates showed lower resistance for ciprofloxacin and gentamicin and the lowest resistance for imipenem and meropenem, except *A. baumannii* isolates that were resistant to all these antibiotics.

The gradually increased resistance for different classes of antibiotics among pathogenic GNB represents a real problem for treatment options which has been reported worldwide. The reason is possibly returning to repeated exposure to penicillin and third generation cephalosporin [15]. There are several resistance mechanisms that are commonly detected in GNB which confer resistance against various antibiotics in clinical use, including the excessive production of extended spectrum β- lactamases and wide dissemination of carbapenemases. These confer resistance not only against β- lactam antibiotics, but also against non β- lactam agents [29]. Along with these mechanisms, loss of porin expression, overproduction of efflux pumps, horizontal gene transfer of mobile genetic elements and significant frequency of mutations within resistance genes can play an essential role in the development of GNB resistance [29].

The MDR pattern was noticed at high rates among the studied GNB isolates, in which all isolates of *K. pneumoniae* 23 (100%) and *A. baumannii* 8 (100%) were MDR. Likewise, 18 (85%) and 12 (66%) of *E. coli* and *P. aeruginosa* respectively showed MDR pattern. Other studies [14, 17, 20, 30, 31, 32] observed that their GNB showed high MDR pattern. The high MDR pattern rates that were demonstrated in GNB isolates could be explained as a result of higher selection pressure created by self-medication, misuse and empirical use of third generation cephalosporin and carbapenems, and the absence of careful monitoring of inhabitant MDR GNB isolates in clinical settings [29].

Random amplification of polymorphic DNA (RAPD) amplification of *K. pneumoniae* yielded 11 distinct patterns with molecular weight 500-3500 bp (Figures 1 and 2). The most frequent bands were 750, 800 and 2500 bp. Whereas, 12 (52%) of isolates showed 1 to 8 bands, but 11 (48%) isolates did not. RAPD amplification of *E. coli* produced 12 distinct patterns with molecular weight of 300-4000 bp (Figures 2 and 3). The most frequent bands were 500, 1000 and 1200 bp. Moreover, 20 (95%) of isolates showed 4 to 10 bands, except 1 (5%) isolate that did not show any band. RAPD amplification of *P. aeruginosa* showed 14 distinct patterns with molecular weight of 400-5000 bp (Figures 3 and 4). The most frequent bands were 750, 800 and 1500 bp. Furthermore, 16 (89%) of isolates showed 3 to 10 bands, except 2 (11%) isolates that did not show any band. RAPD amplification of *A. baumannii* showed 9 distinct patterns with molecular weight of 500-6000 bp (Figure 4). The most frequent band was 4000 bp. Besides, 3 (37%) of isolates showed 1 to 9 bands, whereas 5 (63%) isolates did not show any band.

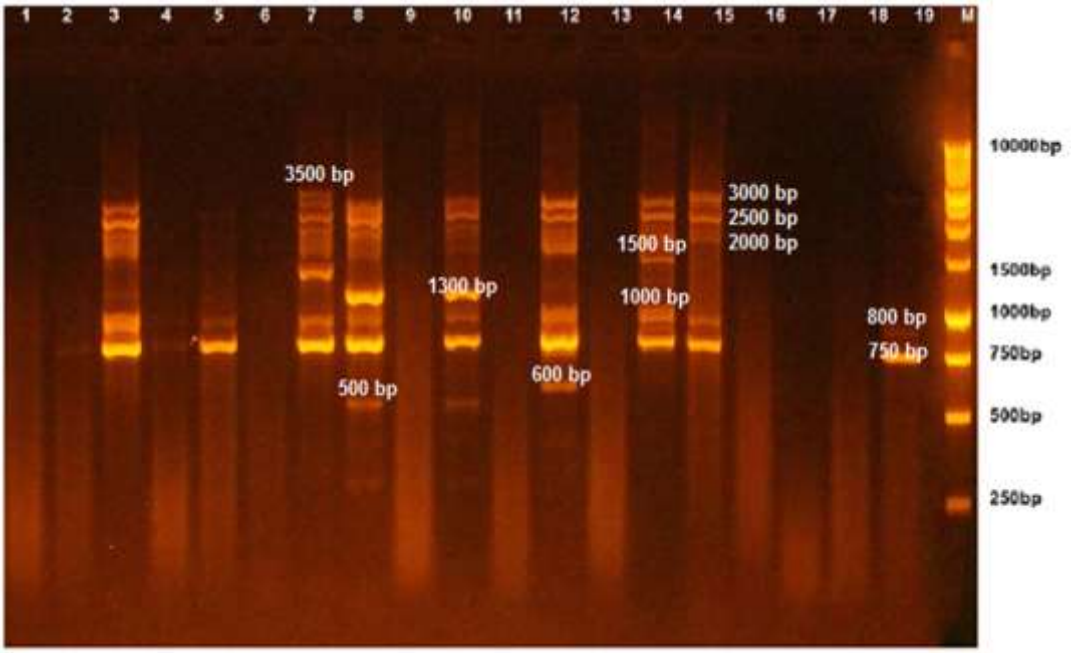

Figure 1: RAPD PCR amplification patterns using 272 primer for *Klebsiella pneumoniae* isolates. M lane: DNA ladder (250-10000bp). The electrophoresis was done using 2% agarose gel at 50V for 100min.

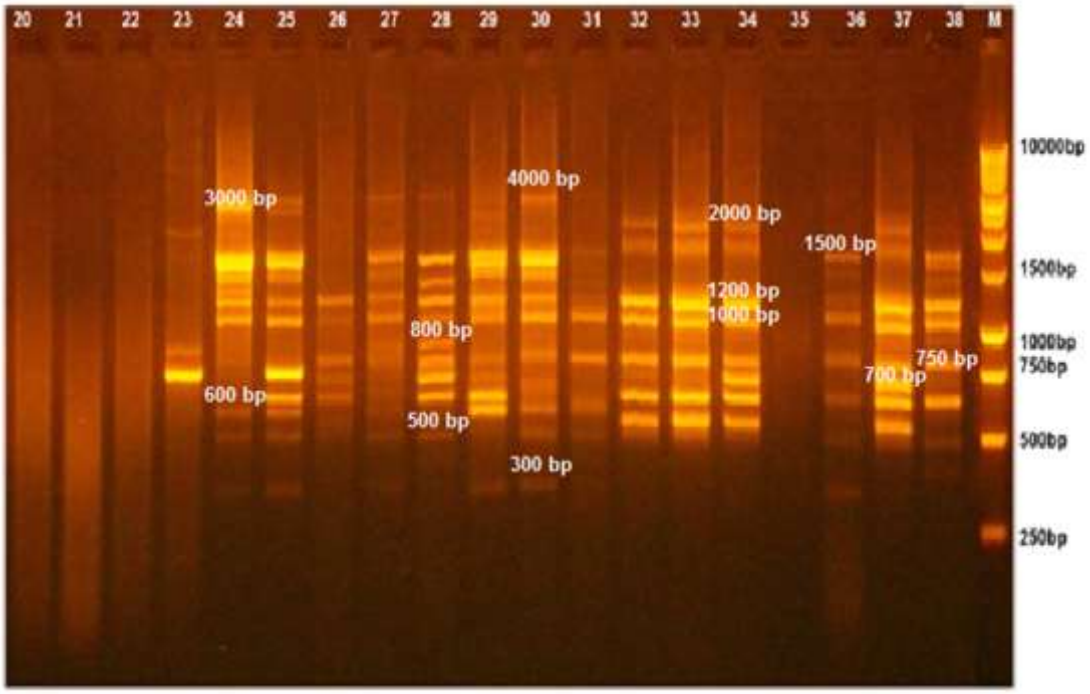

Figure 2: RAPD PCR amplification patterns using 272 primer for *Klebsiella pneumoniae*lane (20-23) and *Escherichia coli* lane (23-38) isolates. M lane: DNA ladder (250-10000bp). Electrophoresis was done using 2% agarose gel at 50V for 100min.

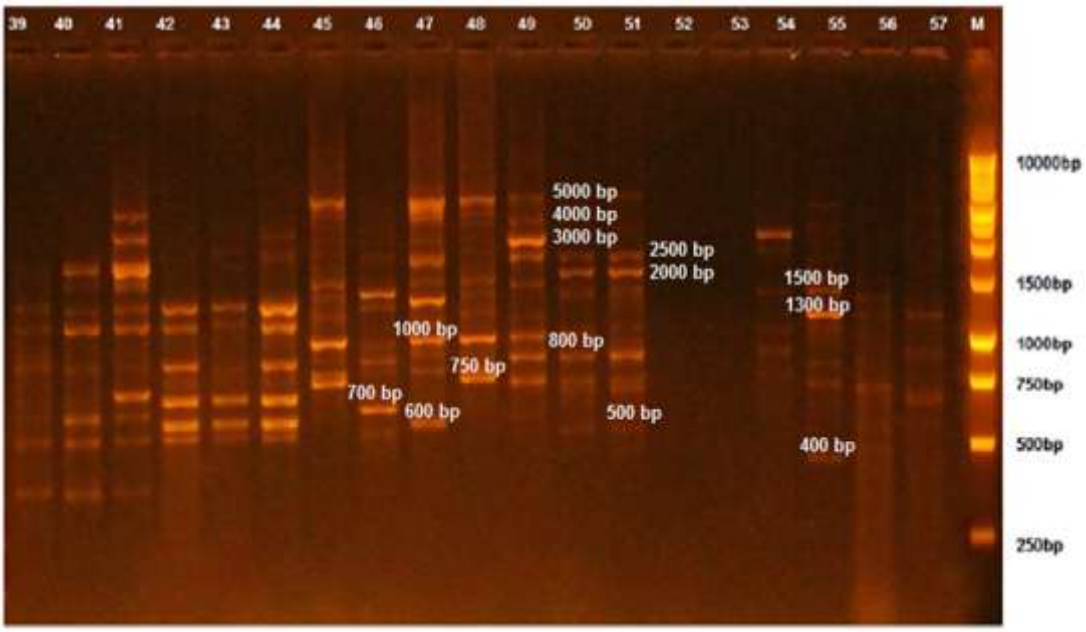

Figure 3: RAPD PCR amplification patterns using 272 primer for *Escherichia coli* lane (39-44) and *Pseudomonas aeruginosa* lane (45-62) isolates. M lane: DNA ladder (250-10000bp). The electrophoresis was done using 2% agarose gel at 50V. for 100min.

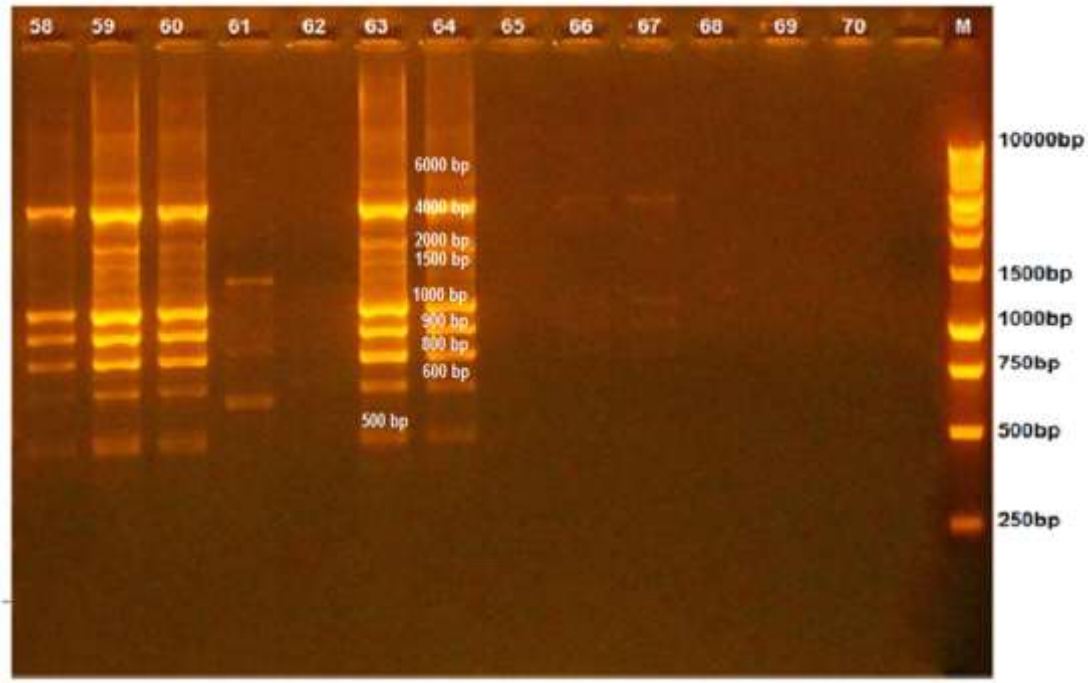

Figure 4: RAPD PCR amplification patterns using 272 primer for *Pseudomonas aeruginosa* lane (58-62) and *Acinetobacter baumannii* lane (63-70) isolates. M lane: DNA ladder (250-10000bp). Electrophoresis was done using 2% agarose gel at 50V for 100min.

Random amplification of polymorphic DNA (RAPD) dendrogram of *K. pneumoniae* revealed the presence of two main clusters at similarity 0.2 (Figure 5). The first cluster contained the isolate 8, whereas the second cluster was the major one which contained 21 isolates arranged into four subclusters: (10, 3), (12, 14), (1, 6, 9, 11, 13, 16, 17, 18, 20, 21, 22, 2, 4) and (19, 23). The isolates 5, 15, and 7 represented distinct genotypic patterns. RAPD dendrogram of *E. coli* showed the presence of three main clusters at similarity 0.2 (Figure 6).

The first cluster involved seven isolates (26, 35, 39, 40, 42, 44, 43). The second cluster involved eight isolates (33, 34, 37, 36, 38, 25, 30, 27). The third cluster involved five isolates (41, 31, 32, 24, 28). Isolate 29 represented a distinct genotypic pattern. RAPD dendrogram of *P. aeruginosa* exhibited the presence of three main clusters at similarity 0.2 (Figure 7). The first cluster encompassed four isolates (45, 48, 46, 56). The second cluster contained ten isolates (59, 61, 57, 52, 62, 53, 54, 55, 58, 60). The third cluster contained three isolates (50, 51, 47). Isolate 49 represented a distinct genotypic pattern. RAPD dendrogram of *A. baumannii* showed the presence of two main clusters at similarity 0.1 (Figure 8). The first cluster comprised two isolates (63, 64), while the second cluster contained six isolates (65, 66, 68, 69, 70, 67).

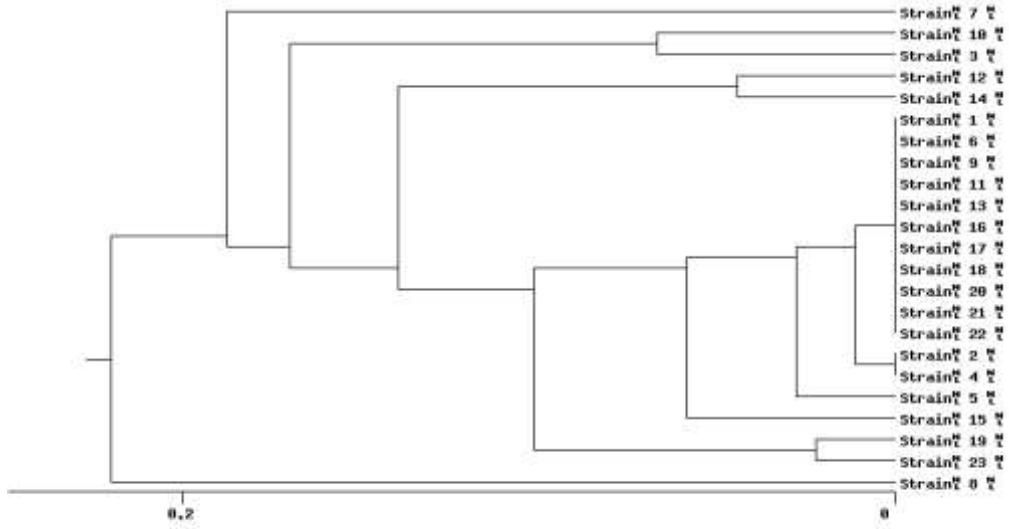

Figure 5: RAPD dendrogram of *Klebsiella pneumoniae.*

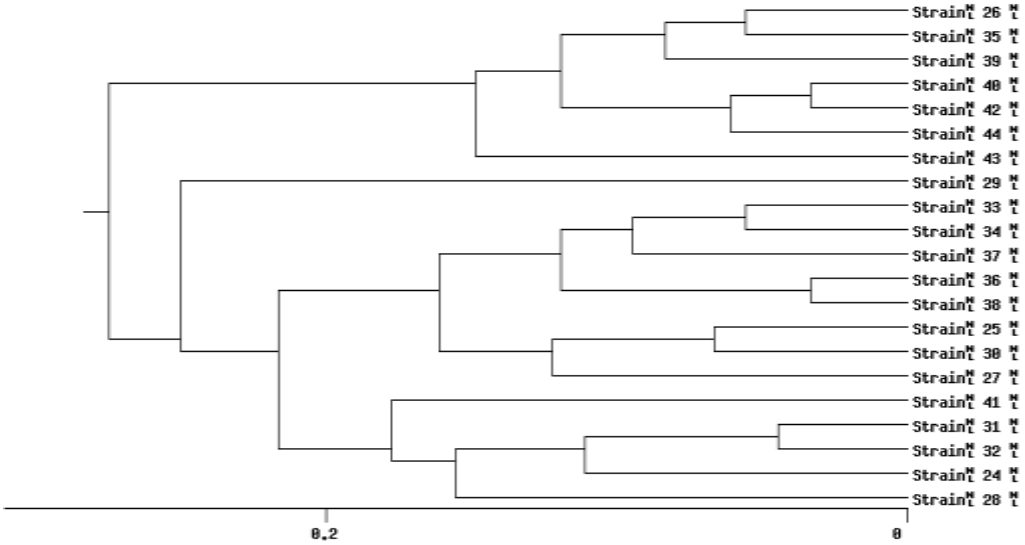

Figure 6: RAPD dendrogram of *Escherichia coli.*

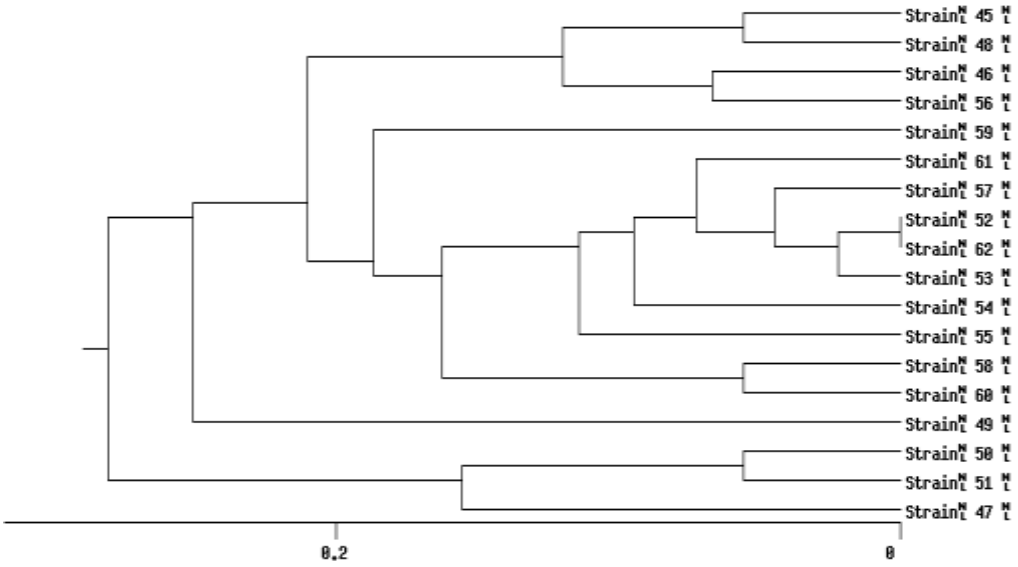

Figure 7: RAPD dendrogram of *Pseudomonas aeruginosa*.

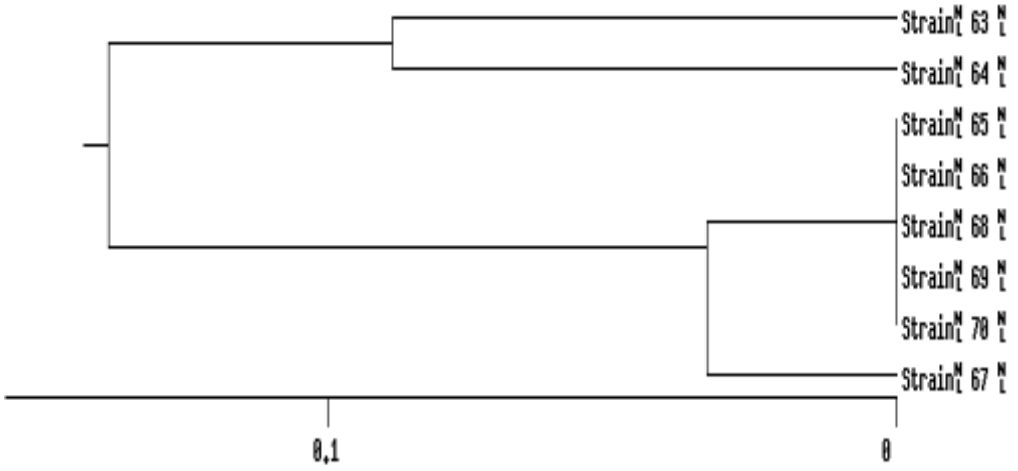

Figure 8: RAPD dendrogram of *Acinetobacter baumannii*.

The results indicated that some isolates grouped in one clone, referring that these isolates had the same epidemiologic routes. These cloned isolates may have been substantially disseminated among patients hospitalized in the same ward or center, especially this study was single center. In contrast, some isolates were untypeable, constituting distinct genotypic patterns. The absence of clonality among some isolates may indicate that these isolates could be endemic in their epidemiologic routes and could have recovered from different sources or were acquired from different environment, referring to genetic diversity of isolates [32].

The correlation between antibiotic resistance patterns and RAPD patterns revealed that certain resistance patterns were associated with most or all RAPD patterns (Tables 8, 9, 10 and 11). Interestingly, the resistance patterns (A1, A2), (A1, A2, A4, A8), (A1, A2, A6) and A1 of *K. pneumoniae*, *E. coli*, *P. aeruginosa* and *A. baumannii* were found to be more frequent among RAPD patterns. This clearly suggests the common source of infections of GNB in the hospital and confirms that cross acquisition or horizontal transmission of GNB can play a pivotal role in the epidemiology of nosocomial colonization and infection with GNB at single study center. Interestingly, most isolates of GNB that belonged to the above

mentioned resistance patterns, were at high risk because their high resistance for most or all of the selected antibiotics. Therefore, there is an urgent need for continuous revision of management and treatment policy to decrease the burden of these strains. Simultaneously, different distribution of RAPD genotypes has been shown in several studies form different countries [33, 34, 35, 36].

Table 8: RAPD patterns of *Klebsiella pneumoniae* isolates

RAPD Pattern	M.W. (bp)	Isolates n (%)	Resistance Pattern
RAPD1	500	2 (8.69)	A1
RAPD2	600	1 (4.34)	A1
RAPD3	750	12 (52.17)	A1, A2, A5, A6
RAPD4	800	10 (43.47)	A1, A2, A5
RAPD5	1000	8 (34.78)	A1, A2, A6
RAPD6	1300	2 (8.69)	A1
RAPD7	1500	4 (17.39)	A1, A2
RAPD8	2000	7 (30.43)	A1, A2, A6
RAPD9	2500	9 (39.13)	A1, A2, A6
RAPD10	3000	7 (30.43)	A1, A2, A6
RAPD11	3500	2 (8.69)	A1

RAPD: random amplification of polymorphic DNA, M.W.: molecular weight, bp: base pair, A: Antibiogram.

Table 9: RAPD patterns of *Escherichia coli* isolates.

RAPD Pattern	M.W. (bp)	Isolates n (%)	Resistance Pattern
RAPD1	300	9 (42.85)	A1, A2, A4, A5, A6, A8
RAPD2	500	20 (95.23)	A1, A2, A3, A4, A5, A6, A7, A8
RAPD3	600	14 (66.66)	A1, A2, A4, A5, A6, A8
RAPD4	700	12 (57.14)	A1, A2, A3, A4, A5, A8
RAPD5	750	13 (61.90)	A1, A2, A3, A4, A5, A6, A7, A8
RAPD6	800	7 (33.33)	A1, A2, A8
RAPD7	1000	20 (95.23)	A1, A2, A3, A4, A5, A6, A7, A8
RAPD8	1200	19 (90.47)	A1, A2, A3, A4, A5, A6, A7, A8
RAPD9	1500	10 (47.61)	A1, A2, A4, A5, A6, A7, A8
RAPD10	2000	9 (42.85)	A1, A2, A3, A4, A5
RAPD11	3000	5 (23.80)	A1, A2, A3, A4
RAPD12	4000	5 (23.80)	A1, A4, A8

RAPD: random amplification of polymorphic DNA, M.W.: molecular weight, bp: base pair, A: Antibiogram.

Table 10: RAPD patterns of *Pseudomonas aeruginosa* isolates

RAPD Pattern	M.W. (bp)	Isolates n (%)	Resistance Pattern
RAPD1	400	1 (5.55)	A6
RAPD2	500	7 (38.88)	A1, A3, A4, A6
RAPD3	600	2 (11.11)	A2, A6
RAPD4	700	6 (33.33)	A1, A2, A4, A6
RAPD5	750	15 (83.33)	A1, A2, A3, A4, A6
RAPD6	800	11 (61.11)	A1, A2, A3, A6
RAPD7	1000	9 (50)	A1, A2. A3, A6
RAPD8	1300	7 (38.88)	A1, A2, A4, A6
RAPD9	1500	13 (72.22)	A1, A2, A3, A4, A6
RAPD10	2000	10 (55.55)	A1, A2, A3, A6
RAPD11	2500	8 (44.44)	A1, A2, A3, A6
RAPD12	3000	4 (2.11)	A1, A2, A6
RAPD13	4000	5 (27.77)	A1, A2, A6
RAPD14	5000	6 (33.33)	A1, A2, A3, A6

RAPD: random amplification of polymorphic DNA, M.W.: molecular weight, bp: base pair, A: Antibiogram.

Table 11: RAPD patterns of *Acinetobacter baumannii* isolates

RAPD Pattern	M.W. (bp)	Isolates n (%)	Resistance Pattern
RAPD1	500	2 (25)	A1
RAPD2	600	2 (25)	A1
RAPD3	800	2 (25)	A1
RAPD4	900	3 (37.5)	A1, A2
RAPD5	1000	3 (37.5)	A1, A2
RAPD6	1500	2 (25)	A1
RAPD7	2000	2 (25)	A1
RAPD8	4000	4 (50)	A1, A2
RAPD9	6000	2 (25)	A1

RAPD: random amplification of polymorphic DNA, M.W.: molecular weight, bp: base pair, A: Antibiogram.

Conclusion

The high levels of CRP, PCT and LBP recorded in acute leukemia patients could refer to the induction of the inflammatory response stimulated by GNB infections, aiding in early prediction of infection to optimize clinical treatment in these risk population. The genetic variations in RAPD patterns and dissemination of epidemiologically related clones among GNB highlight the need for increased awareness of these pathogenic patterns and demand the control and prevention of nosocomial outbreak caused by GNB infections, thus improving the hygiene of patients and decreasing the hospitalized period.

References

[1] R. H. Abdulridha, N. K. Jawad and A. T. Numan, "Prevalence and Risk of Leukemia Reported Cases, Observational Descriptive Statistic from Iraqi Center for Hematology in Baghdad Province", *Indian Journal of Forensic Medicine and Toxicology*, vol. 15, no. 1, pp. 2428-2433, 2021.

[2] R. Nayak and S. Rai, *Rapid Review of Hematology*, New Delhi :Jaypee Brothers Medical Publishers (P) Ltd, 2014, p. 140.

[3] T. Staudinger and F. Pène, "Current insights into severe sepsis in cancer patients", *Revista Brasileira de Terapica Intensiva*. vol. 26, no. 4, pp. 335-338, 2014.

[4] K. Arıkan, E. Karadağ-Oncel, S. Aytac, A. B. Cengiz, F. Duygu Cetinkaya, A. Kara and M. Ceyhan, "The use of serum endothelial adhesion molecules in pediatric patients with leukemia with febrile neutropenia to predict bacteremia", *Cytokine*, vol. 148, 155692, 2021.

[5] Y. Zhang, M. La, J. Sun, M. Chen, D. Liu, X. Liu and Y. Kang, "Diagnostic Value and Prognostic Significance of Procalcitonin Combined with C-Reactive Protein in Patients with Bacterial Bloodstream Infection", *Computational and Mathematical Methods in Medicine*, vol. 2022, 6989229, 2022.

[6] P. Liang and F. Yu, "Value of CRP, PCT, and NLR in prediction of severity and prognosis of patients with bloodstream infections and sepsis", *Frontiers in Surgery*, vol. 9, 857218, 2022.

[7] A. D. Irwin and E. D. Carrol, "Procalcitonin", *Archives of Disease in Childhood: Education and Practice Edition*, vol. 96, no. 6, pp. 228-233, 2011.

[8] J. Zweigner, R. R. Schumann and J. R. Wener, "The role of lipopolysaccharide binding protein in modulating the innate immune response", *Microbes and Infection*, vol. 8, no. 3, pp. 946–952, 2006.

[9] F. Nanvazadeh, A. D. Khosravi, M. R. Zolfaghari and N. Parhizgari, "Genotyping of *Pseudomonas aeruginosa* strains isolated from burn patients by RAPD-PCR" *Burns*, vol. 39, no. 7, pp. 1409-1413, 2013.

[10] P. M. Tille, *Bailey and Scott's Diagnostic Microbiology*, China: Elsevier, 2017, p. 1115.

[11] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 32nd ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2022.

[12] F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J.G., Seidman, J.A. Smith and K. Struhl, *Current Protocols in Molecular Biology*, USA: Wiley and Sons, Inc., 2003.

[13] A. Hematzadeh and M. Haghkhah, "Biotyping of isolates of *Pseudomonas aeruginosa* isolated from human infections by RAPD and ERIC-PCR", *Heliyon*, vol. 7, no. 9, e07967, 2021.

[14] S. Mamishi, S. Mahmoudi, N. Naserzadeh, R. Hosseinpour Sadeghi, M. T. Haghi Ashtiani, A. Bahador, M. R. Abdosalehi, M. Rahmani and B. Pourakbari, "Antibiotic resistance and genotyping of gram-negative bacteria causing hospital-acquired infection in patients referred to Children's Medical Center", *Infection and drug resistance*, vol. 12, pp. 3377–3384, 2019.

[15] H. S. Elbadawi, K. M. Elhag, E. Mahgoub, H. N. Altayb, F. Ntoumi, L. Elton, T. D. McHugh, J. Tembo, G. Ippolito, A. Y. Osman, A. Zumla, M. M. A. Hamid, "Detection and characterization of carbapenem resistant Gram-negative bacilli isolates recovered from hospitalized patients at Soba University Hospital, Sudan", *BMC Microbiology*, vol. 21, no. 136, 2021.

[16] H. S. Abdul-Mohammed, A. K. Mohammed and Z. M. Ahmed, "Imipenem Resistance in Gram-Negative Bacteria in the Central Pediatric Teaching Hospital in Baghdad, Iraq", *Archives of Razi Institute*, vol. 77, no. 1, pp. 123-128, 2022.

[17] N. A. Kamel, W. N. El-Tayeb, M. R. El-Ansary, M. T. Mansour and K. M. Aboshanab. "Phenotypic screening and molecular characterization of carbapenemase-producing Gram-negative bacilli recovered from febrile neutropenic pediatric cancer patients in Egypt", *PLoS ONE*, vol. 13, no. 8, e0202119, 2018.

[18] H. S. A. Al-Rawazq, A. A. Hussein and A. K. Mohammed, "Antibiotic Resistance of Isolated Gram Negative Bacilli from Different Clinical Sample in a Central Teaching Hospital of Pediatric in Baghdad", *Journal of Pure and Applied Microbiology*, vol. 13, no. 1, pp. 349-354, 2019.

[19] M. T. Hasan, K. K. Ghaima, A. A. Abdulhassan and M. K. Alwan, "Prevalence and Antimicrobial Susceptibility of Gram-negative Bacteria in Pediatric Patients with Urinary Tract Infections in Baghdad Hospitals, Iraq", *Annals of R.S.C.B.* vol. 25, no. 6, pp. 6102-6109, 2021.

[20] S. H. Haji, S. T. H. Aka and F. A. Ali, "Prevalence and characterisation of carbapenemase encoding genes in multidrug-resistant Gram- negative bacilli", *PLoS ONE*, vol. 16, no. 11, e0259005,2021.

[21] M. Yang, S. J. Choi, J. Lee, D. G. Lee, Y. J. Kim, Y. J. Park and E. J. Oh, "Serum procalcitonin as an independent diagnostic markers of bacteremia in febrile patients with hematologic malignancies", *PLoS ONE*, vol. 14, no. 12, e0225765, 2019.

[22] M. Shokripour, N. Omidifar, K. Salami, M. Moghadamiand B.Samizadeh, "Diagnostic Accuracy of Immunologic Biomarkers for Accurate Diagnosis of Bloodstream Infection in Patients with Malignancy: Procalcitonin in Comparison with C-Reactive Protein", *Canadian Journal of Infectious Diseases and Medical Microbiology*, vol. 2020, 8362109, 2020.

[23] R. Moustafa, T. Albouni and G. Aziz, "The role of procalcitonin and pre-sepsin in the septic febrile neutropenia in acute leukemia patients", *PLoS ONE*, vol. 16, no. 7, e0253842, 2021.

[24] G. Zeng, D. Chen, R. Zhou, X. Zhao, C. Ye, H. Tao, W. Sheng and Y. Wu, "Combination of C-reactive protein, procalcitonin, IL-6, IL-8, and IL-10 for early diagnosis of hyperinflammatory state and organ dysfunction in pediatric sepsis", *Journal of Clinical Laboratory Analysis*, vol. 36, no. 7, e24505, 2022.

[25] M. Pavcnik-Arnol, S. Hojker and M. Derganc, "Lipopolysaccharide-binding protein in critically ill neonates and children with suspected infection: comparison with procalcitonin, interleukin-6, and C-reactive protein", *Intensive Care Medicine*, vol. 30, no. 7, pp. 1454-1460, 2004.

[26] J. Pavare, I. Grope, I. Kalnins and D. Gardovska, "High-mobility group box-1 protein, lipopolysaccharide-binding protein, interleukin-6 and C-reactive protein in children with community acquired infections and bacteraemia: a prospective study", *BMC Infectious Disease*, vol. 10, no. 28, 2010.

[27] L. Kitanovski, J. Jazbee and S. Hojker, "Diagnostic Accuracy of Lipopolysaccharide-Binding Protein for Predicting Bacteremia/Clinical Sepsis in children with Febrile Neutropenia: Comparison with Interleukin-6, Procalcitonin, and C-reactive protein", *Support Care Cancer*, vol. 22, pp. 269-277, 2014.

[28] R. A. H. Al-Lami, H. S. A. Al-Hayanni and Z. H. Shehab, "Molecular Investigation of Some Beta-Lactamase Genes by PCR and DNA Sequencing Techniques in clinical *Escherichia coli*", *Iraqi Journal of Science*, vol. 63, no. 10, pp. 4205-4212, 2022.

[29] F. S. Codjoe, "Detection and characterization of carbapenem-resistant gram-negative bacilli infections in Ghana", Ph.D. Thesis, Sheffield Hallam University, England, p. 283, 2016.

[30] M. S. Mustafa and R. M. Abdullah, "Role of *oqxA* and *oqxB* Genes in the Development of Multidrug Resistant Phenotype among Clinical *Klebsiella pneumoniae* Isolates from Various Cases", *Iraqi Journal of Science*, vol. 61, no. 8, pp. 1902-1912, 2020.

[31] S. A. Ali and H. O. M. Al-Dahmoshi, "Detection of Efflux Pumps Gene and Relation with Antibiotics Resistance in Uropathogenic *Escherichia coli* (UPEC) Isolated from Patients with Cystitis", *Iraqi Journal of Science*, vol. 63, no. 6, pp. 2388-2397, 2022.

[32] N. N. Yaseen and D. A. Ahmed, "Detection of *mex*B Multidrug Efflux Genes in Some Local Isolates of *Pseudomonas aeruginosa*", *Iraqi Journal of Science*, vol. 64, no. 1, pp. 111-118, 2023.

[33] R. S. Sallman, S. S. Hussein and M. R. Ali, "ERIC- PCR Typing, RAPD-PCR Fingerprinting and Quorum Sensing Gene Analysis of *Pseudomonas aeruginosa* Isolated from Different Clinical Sources", *Al-Mustansiriyah Journal of Science*, vol. 29, no. 2, pp. 50-62, 2018.

[34] A. Saadatian Farivar, J. Nowroozi, G. Eslami and A. Sabokbar, "RAPD PCR Profile, Antibiotic Resistance, Prevalence of *armA* Gene, and Detection of KPC Enzyme in Klebsiella pneumoniae Isolates", *The Canadian journal of infectious diseases and medical microbiology*, vol. 2018, 6183162, 2018.

[35] D. A. Mraidi and I. J. Lafta, "RAPD-PCR and phylogenetic analysis of *E. coli* isolated from human and cattle urinary tract infections", *Iraqi Journal of Veterinary Sciences*. vol. 35, Suppl. 3, pp. 59-66, 2021.

[36] R. Abozahra, S. M. Abdelhamid, A. G. Elsheredy, K. E. Abdulwahab and K. Baraka, "Genotyping and Molecular Characterization of Carbapenem-resistant *Acinetobacter baumannii* Strains Isolated from Intensive Care Unit Patients", *Korean Journal of Microbiology and Biotechnology*, vol. 49, no. 2, pp. 239-248, 2021.