

Analysis of Plasmids and Restriction Fragment Length Polymorphisms of *Acinetobacter baumannii* Isolated from Hospitals- AL Jouf Region- KSA

Samy A. Selim and Nashwa I. Hagag

Abstract—The objectives of the current study are to determine the prevalence, etiological agents, drug susceptibility pattern and plasmid profile of *Acinetobacter baumannii* isolates from Hospital-Acquired Infections (HAI) at Community Hospital, Al Jouf Province, Saudi Arabia. A total of 1890 patients had developed infection during hospital admission and were included in the study. Among those who developed nosocomial infections, 15(9.4), 10(2.7) and 118 (12.7) had respiratory tract infection (RTI), blood stream infections (BSI) and urinary tract (UTI) respectively. A total of 268 bacterial isolates were isolated from nosocomial infection. *S. aureus* was reported in 23.5% for of the total isolates followed by *Klebsiella pneumoniae* (17.5%), *E. coli* (17.2%), *P. aeruginosa* (11.9%), coagulase negative staphylococcus (9%), *A. baumannii* (7.1%), *Enterobacter* spp. (3.4%), *Citrobacter freundii* (3%), *Proteus mirabilis* (2.6%), and *Proteus vulgaris* and *Enterococcus faecalis* (0.7%). Isolated organisms are multi-drug resistant, predominantly Gram-positive pathogens with a high incidence of methicillin-resistant *S. aureus*, extended spectrum beta lactamase and vancomycin resistant enterococci organisms. The RFLP (Fragment Length Polymorphisms) patterns of plasmid preparations from isolated *A. baumannii* isolates had altered RFLP patterns, possibly due to the presence of plasmid(s). Five *A. baumannii* isolates harbored plasmids all of which were not less than 2.71kbp in molecular weight. Hence, it showed that the gene coding for the isolates were located on the plasmid DNA while the remaining isolates which have no plasmid might showed gene coding for antibiotic resistance being located on chromosomal DNA. Nosocomial infections represent a current problem in Community Hospital, Al Jouf Province, Saudi Arabia. Problems associated with SSI include infection with multidrug resistant pathogens which are difficult to treat and are associated with increased mortality.

Keywords—Hospital-Acquired Infections, *Acinetobacter baumannii*, antibiotic resistance, plasmid profile, RFLP patterns, Al Jouf Province, Saudi Arabia.

I. INTRODUCTION

THE definition of a hospital acquired infection (HAI), also known as a nosocomial infection, is an infection acquired during hospital care which was not present or incubating at the

time of admission [1]. Nosocomial infections occur worldwide and affect both developed and resource-poor countries. Infections acquired in health care settings are among the major causes of death and increased morbidity among hospitalized patients. They are a significant burden both for the patient and for public health. At any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospital [2]. The highest frequencies of nosocomial infections were reported from hospitals in the Eastern Mediterranean and South-East Asia Regions (11.8 and 10.0% respectively), with a prevalence of 7.7 and 9.0% respectively in the European and Western Pacific Regions [3]. The most frequent nosocomial infections are infections of surgical wounds, urinary tract infections and lower respiratory tract infections. The WHO study, and others, has also shown that the highest prevalence of nosocomial infections occurs in intensive care units and in acute surgical and orthopaedic wards [1].

Epidemiological and etiological characteristics of nosocomial infections show variations among countries and even among different hospitals in the same country. Many of these infections are associated with micro-organisms that are resistant to multiple antibiotics and can easily spread on the hands of personnel [4]. They are important for both patient and public health problem in developing countries, as well as in some developed countries [5].

Plasmids, which are extrachromosomal double-stranded DNA materials, have been found to be useful for pathogens' genetic diversity and prowess as infectious agents. Profiling pathogens for their harbored plasmids has been found to be very useful in epidemiological studies, diagnosis, and elucidation of mechanisms of drug resistance. Plasmids have also been found useful in knowing whether two or more strains of a pathogen evolve from the same microorganism, thereby providing a reliable insight into the genetic relatedness of pathogens in an environment [6].

This study was designed to evaluate the frequency of HAI at Community Hospital, Al Jouf Province, Saudi Arabia in relation to different risks and to determine the main sites of infections, common microorganisms and their antimicrobial susceptibilities. The antibiotic susceptibility and the plasmid profile of the isolated microbes were determined to evaluate whether the gene coding for the resistance is located on the chromosome or plasmid. Also RFLP analysis used to examine a

Samy A. Selim is with the Department of Medical Laboratory Science, College of Applied Medical Science, Al Jouf University, Sakaka, Al Jouf, P.O. 2014, Saudi Arabia and with the Microbiology Section, Faculty of Science, Suez Canal University, Ismailia, 41522, Egypt (e-mail: sadomm2003@yahoo.com).

Nashwa I. Hagag is with the Microbiology Section, Faculty of Science, Suez Canal University, Ismailia, 41522, Egypt and with the Department of Human and Animal Biology, Università degli Studi di Torino, Torino, Italy.

subset of *Acinetobacter baumannii* isolates from Al Jouf Region for additional plasmids.

II. MATERIALS AND METHODS

A. Selection and Evaluation of Study Subjects

To select the target groups convenient sampling technique was employed. Thus, a careful clinical examination was conducted by physician to all adult patients who were admitted to surgical wards and general intensive care unit (ICU). Examination of wounds and catheter entry sites, review of procedures that might led to infection were made. This was done in order to exclude community-acquired infections and to determine any underlying risk factors.

B. Patients

The study was conducted to all patients admitted to surgical wards and general ICU at Community Hospitals, Al Jouf Province, Saudi Arabia. The hospitals receive referred patients from all parts of the province and provide local emergency service. All patients admitted at different adult ICU were monitored daily by attending physicians for subsequent development of nosocomial, which must meet at least one of the following criteria: Criterion 1: Patient has a recognized pathogen cultured from one or more specimens cultures and the organism cultured from specimens is not related to an infection at another site. Criterion 2: Patient has at least one of the following signs or symptoms: fever ($>38^{\circ}\text{C}$), chills, or hypotension and at least one of the following: Common skin contaminant (e.g., diphtheroids, *Bacillus* sp., coagulase-negative staphylococci, or micrococci) is cultured from two or more specimens' cultures drawn on separate occasions. The Signs and symptoms of infection appear 48 hours to four days after admission, and there are no signs or symptoms of infection at the time of admission, proven by history and clinical examination [7].

C. Samples Collection and Processing

During the study, all adult (>15 years) patients admitted to surgical wards and general ICU with suspected nosocomial infection were included. Specimens were collected from patients admitted to the surgical wards and general ICU and suspected of developing nosocomial infection based on their clinical findings. The specimens were collected using standard procedures and analyzed accordingly. Blood sample were drawn aseptically when bloodstream infection was suspected (adult patients who had chills or fever). About 10ml of venous blood was collected and immediately inoculate directly in to a tube containing thioglycollate broth. Blood was incubated aerobically at 37°C for 10 days and checked for turbidity as an indication of growth. Urine sample was collected for bacteriological examination by the mid stream method or catheterization into sterilized container and cultured before and after using catheter. Urine sample which did not show significant growth ($<0.4\text{CFU/ml}$ of urine) before the insertion of catheter and urine culture with colony count $\geq 105\text{CFU/ml}$ of urine after catheterization were considered indicative of significant infection. Wound infections occur as complications

of surgery, trauma, or disease that interrupts a skin surface. Material from infected wounds was collected aseptically by using sterile swab and culture, gram stain and biochemical test done respectively to isolate the causative agents.

D. Identification of Bacterial Isolates

Identification of gram negative bacilli: gram negative bacilli were identified by API 20E system (Biomérieux SA, Montalieu Vercieu and France). Identification of enterococcal isolates: suspected enterococcal isolates on bile esculin azide agar were identified by the Colony morphology, Gram staining, the catalase test and the PYR test (production of pyrrolidonyl arylamidase). Identification of staphylococcal isolates: according to Louie et al. [8], staphylococci were identified by standard methods including the gram stain, catalase test and tube coagulase test. Samples were cultured on Mannitol Salt Agar (MSA) (Oxoid, UK), where *S. aureus* produces yellow colonies (1mm in diameter) surrounded by a yellow medium, and CNS forms small orange colonies surrounded by a red or purple medium [9]. Isolates which showed positive growth on Mannitol Salt Agar plates were subcultured on an Oxacillin Resistant Screening Agar Base (ORSAB) medium (Oxoid, UK) for Detection of oxacillin resistance.

E. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of isolates was tested by the disk diffusion method according to the National Committee on Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards [10] recommendations, using Mueller—Hinton medium.

F. Plasmid Analysis

Plasmid DNA was isolated from the 7 strains of *A. baumannii* by the alkaline method of Zhou et al. [11], with some modifications. Cells were grown overnight in 3ml of LB broth medium at 37°C and 1.5ml was spun down at $10,000\times g$ for 1min. The pellet obtained was washed in 1ml of TE buffer (50mM Tris-HCl, 10mM EDTA - pH 8.0). After centrifugation the cells were resuspended in 50ml of TE, and 400ml of lysing solution (50mM Tris and 3% SDS - pH 12.45) were added. The solution was mixed by brief agitation and incubated in a water bath at 60°C for 30min. The lysate was subsequently incubated on ice water, and 20ml of 2M Tris-HCl (pH 7.0) were added. The extraction was made by addition of an equal volume of phenol-chloroform solution. After centrifugation as above for 10min, 60ml of aqueous phase were mixed with loading buffer and applied to a vertical agarose gel (0.7%) for electrophoresis at 60V. Gels were stained with ethidium bromide and photographed under a UV lamp (Gel Documentation System, BioRad Company, USA).

G. Determination of Molecular Weight of Plasmids and Plasmid Type

Molecular weights of plasmids from different *A. baumannii* strains were determined by using the molecular weight determination parameter in Gel documentation system (BioRad Company, USA) with plasmids pUC19 (Promega, USA) used as comparative standards based on triplicate observations.

Average of three readings was recorded as final molecular weight. 1 kb DNA extension ladder and DNA Mass Ladder (Promega, USA) were also run. A plasmid type was defined as any plasmid pattern which varied from another pattern with regard to number and size of plasmids.

H. RFLP and Restriction Enzyme Analysis of Plasmid DNA

Once the plasmid DNA was extracted, it was digested using DNA restriction enzymes. The enzymes *Bam*HI, *Eco*RI, *Hind*III, *Hae*III and *Sma*I were employed to examine RFLP patterns of extrachromosomal DNA of each isolate. For the restriction digest reactions, 15µl of the plasmid template was used in combination with 2µl 10X React3 buffer, 2µl 10mM RNase and 1µl restriction endonuclease enzymes (Promega, USA). This reaction was incubated at 37°C for two hours to allow sufficient digestion. Fragments from the digested DNA were separated with gel electrophoresis in 1.0% agarose containing ethidium bromide and visualized with a UV transilluminator.

I. Statistical Analysis

Infection rates were calculated as overall infection rate and incidence density rate. The Chi-square test was used to compare the frequency of infections. Data were analyzed using SPSS version 16 for windows.

III. RESULTS

One thousand two hundreds ninety two patients admitted in surgical ward and general ICU at Community Hospitals, Al Jouf Province, Saudi Arabia were studied for prevalence of nosocomial infections. On admission, they were carefully examined clinically to exclude community-acquired infections and to determine any underlying risk factors. Concerning the wounds, 337 (77.5%) were classified as clean and 98 (22.5%) as contaminated. Antibiotic prophylaxis was given to 78% of the patients before sample collection. Among 1890 patients, 241 (12.7%) were confirmed to have nosocomial infection. The distribution of nosocomial infection among positive cases presented in Table I indicates that 15(9.4) were respiratory tract infection, 10 (2.7%) blood stream infections, 98 (22.5) surgical site infections and 118 (12.7%) urinary tract infections. Surgical procedures, insertion of urinary catheter, insertion of central venous catheter and mechanical ventilation were significantly associated with nosocomial infection ($p < 0.05$) in this study.

TABLE I
DIAGNOSIS AND DISTRIBUTION OF PATIENTS WITH NOSOCOMIAL INFECTIONS

Type of infection	No. of Studied cases	Infected (%)
Respiratory Tract infection	160	15 (9.4)
Bloodstream infection	365	10 (2.7)
Surgical site infection	435	98 (22.5)
Urinary Tract infection	930	118 (12.7)
Total	1890	241(12.8)

A total of 268 bacterial isolates were isolated from nosocomial infection (n=241) cases. *S. aureus* accounted for

23.5% of the total isolates followed by *Klebsiella pneumoniae* (17.5%), *E. coli* (17.2%), *P. aeruginosa* (11.9%), *coagulase negative staphylococcus* (9%), *A. baumannii* (7.1%), *Enterobacter* spp. (3.4%), *Citrobacter freundii* (3%), *Proteus mirabilis* (2.6%) and *P. vulgaris* and *Enterococcus faecalis* (0.7%) (Table II). The Gram-positive and negative bacteria accounted for 93/272(34.2%) and 179/272(65.8 %), respectively ($p < 0.05$). Among surgical site infection (n = 125), *S. aureus* (34.4%), *K. pneumoniae* (17.6%) and *P. aeruginosa* (14.4%) were isolated. Among UTI (n=118), *E. coli* (30.5%), *S. aureus* (14.4%) and *K. pneumoniae* (13.6%); and in BSI (n=12) *S. aureus* (41.7%), *coagulase negative staphylococcus* (33.3%) followed by *P. aeruginosa* (16.6%) were the commonest bacterial pathogens isolated. While in RTI (n=17) *K. pneumoniae* represented dominant strain (47.1%) of the total isolates (Table II).

TABLE II
DISTRIBUTION OF BACTERIAL ISOLATES ASSOCIATED WITH NOSOCOMIAL INFECTION RECORDED DURING HOSPITAL-ACQUIRED SURVEILLANCE

Pathogen	RTI n =17	BSI n=12	SSI n =125	UTI n =118	Total (%)
Gram negative					
<i>Acinetobacter</i> spp	0	0	0	10	19(7.1)
<i>Citrobacter freundii</i>	0	0	0	8	8(3)
<i>Escherichia coli</i>	0	0	10	36	46(17.2)
<i>Enterobacter</i> spp	0	0	0	9	9(3.4)
<i>Klebsiella pneumoniae</i>	8	1	22	16	47(17.5)
<i>Proteus mirabilis</i>	3	0	0	4	7(2.6)
<i>Proteus vulgaris</i>	0	0	9	2	2(0.7)
<i>Pseudomonas aeruginosa</i>	4	2	18	8	32(11.9)
<i>Serratia marcescens</i>	0	0	6	3	9(3.4)
Gram positive					
CNS*	0	4	15	5	24(9)
<i>Staphylococcus aureus</i>	2	5	43	17	67(23.5)
<i>Enterococcus faecalis</i>	0	0	2	0	2(0.7)

Where; RTI: Respiratory Tract infection, BSI: Bloodstream infection, SSI: Surgical Site infection, UTI: Urinary Tract infection and CNS: Coagulase-negative staphylococci

The susceptibility patterns of gram-positive bacteria (n=37) isolated from nosocomial infection against 14 antimicrobial agents are presented in Table III. Almost all bacteria isolates showed multiple drug resistance (resistance to two or more drugs). Most isolates showed high level of resistance (100%) to ampicillin, tetracycline and chloramphenicol; and >80% (high level of resistance) to amoxicillin- clavulanate and streptomycin. But other studied antimicrobial agents showed low level of resistance (<60%). The susceptibility patterns of gram-negative bacteria (n=50) isolated from nosocomial infections against 13 antimicrobial agents are presented in Table III. All isolates showed high level of resistance (100%) to ampicillin, Tetracycline; and > 80% (high level of resistance) to chloramphenicol. Like Gram positive bacteria, almost all isolated Gram negative bacteria showed multi-drug resistance.

Fig. 1 showed the plasmid profile patterns of 7 *A. baumannii* strains isolated from Al Jouf region hospital. Five contained plasmids with molecular weight of 2.71kb. Plasmids were not detected in other two strains. When digested with *Hind* III

plasmids extracted of different *A. baumannii* isolates shared the same restriction pattern, with three bands of almost identical sizes (Fig. 2). Digestion of the plasmids with *EcoRI* generated distinctive restriction profiles for plasmids: two fragments 181bp and 434bp (Fig. 3). Other tested restriction enzymes were unaffected on plasmids, including *BamHI*, *HaeIII* and *SmaI*. Therefore, two separate enzymatic digestions produced species-specific RFLP profiles for bacterial isolates.

TABLE III
FREQUENCY OF ANTIMICROBIAL RESISTANCE PATTERNS OF GRAM-POSITIVE AND GRAM-NEGATIVE ISOLATES

Antimicrobial Agents	Gram-positive				Gram-negative		
	<i>E. faecalis</i> (n=12)	<i>S. aureus</i> (n=5)	CNS* (n=10)	<i>P. vulgaris</i> (n=8)	<i>E. coli</i> (n=20)	<i>K. pneumoniae</i> (n=15)	<i>P. aeruginosa</i> (n=7)
Augmentin	100	90	100	90	60	60	80
Ampicillin	100	100	100	100	100	100	100
Azithromycin	ND	ND	ND	100	100	90	75
Chloramphenicol	100	100	100	90	80	80	100
Ciprofloxacin	90	43	25	30	66	70	40
Erythromycin	100	14	20	0	0	0	0
Cefazolin	50	21	60	30	40	25	0
Gentamicin	35	38	0	30	28	10	50
Methicillin	100	15	100	ND	ND	ND	ND
Nitrofurantoin	100	100	0	60	40	80	90
Norfloxacin	70	50	0	40	50	80	50
Rifampicin	20	45	50	0	10	0	0
Streptomycin	100	90	100	50	100	80	85
Tetracycline	100	100	100	0	15	20	90
Vancomycin	0	0	0	ND	ND	ND	ND

*Coagulase-negative staphylococci, ND: Not Determine.

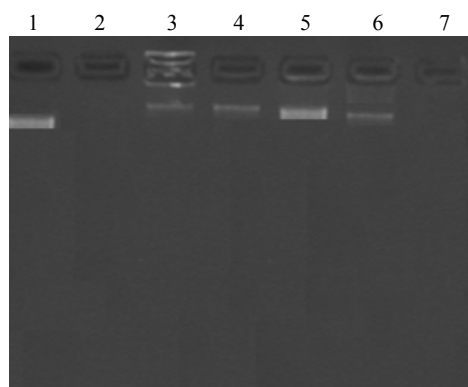


Fig. 1 Plasmid DNA profile of 7 *A. baumannii* isolates

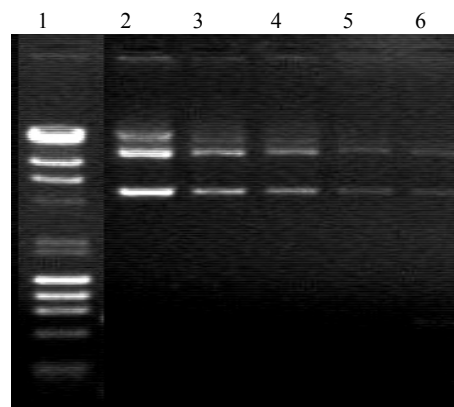


Fig. 2 Restriction digestion of plasmid products of *A. baumannii* strains with *Hind III*: where Lane 1: 100-bp ladder

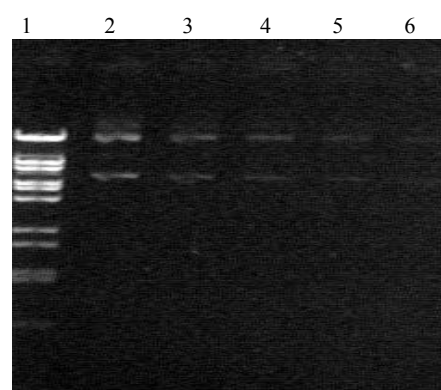


Fig. 3 Restriction digestion of plasmid products of *A. baumannii* strains with *EcoRI*: where Lane 1: 100-bp ladder

IV. DISCUSSION

Nosocomial infections (NIs) are becoming increasing problems for hospitalized patients [12]. They are major causes of death and disability worldwide. According to estimates reported by the World Health Organization, up to 15% of hospitalized patients suffer from infections associated with health care [13]. Moreover, hospitals worldwide are continuing to face the crisis of the upsurge and dissemination of antimicrobial-resistant bacteria, particularly those causing nosocomial infections in ICU patients [14]. In this study, the overall prevalence of nosocomial infection in surgical ward and general ICU at Community Hospital, Al Jouf Province, Saudi Arabia is (12.8%), which is lower than the other studies in the different hospital in Saudi Arabia, which were 48.3% [15]. This decrease in the prevalence of nosocomial infections in this hospital may paralleled to paying attention to well-established processes for decontamination and cleaning of soiled instruments and other items, followed by sterilization and high-level disinfection processes and improving safety in operating rooms and other high-risk areas where the most serious and frequent injuries and exposures to infectious agents occur. Another factor that can account for the lower infection rate may be due to the availability of high number of health

personnel and setup of the hospital. Other possible reason may be during the study period there was a new method for elected cases from the emergence operated immediately within two days. This short period of exposure before operation may contribute to low prevalence rate of nosocomial infection in surgical wards. Besides these, the current study showed lower prevalence of nosocomial infection than reports from different countries like Tunisia which was 13% [16], Kosova 17.4% [17] and Morocco 17.8% [18]. One reason for this lower prevalence may due to the method of sample analysis, which was dependent mainly on bacteriological agents. Anaerobic bacterial infections are not included since they are also other causative agents of nosocomial infection. On the other hand most of the study in the above mentioned countries included anaerobic bacteria, fungi and viruses in their study.

In this study, the most nosocomial infection was found to be surgical site infection. Since all patients were exposed for surgical procedures, there is an increase risk of getting an infection in the hospital by direct invading of the patient's body, gives bacteria a way in to normal sterile parts of the body. This infection can be acquired from contaminated surgical equipment or from health care workers. Also the susceptibility to surgical wound infections were enhanced by poor wound care and prolonged hospitalization. Due to the above reason, surgical wound can colonized by micro-organisms and showed the highest nosocomial infection site. Urinary tract infection was the second infection site in the present study. Since, all of the patients with nosocomial UTI had urinary catheters and catheterization increased the rate of infection. There may be bacteria in or around the urethra but they normally cannot enter to the bladder. A catheter can pick up bacteria from the urethra and allow them into the bladder, causing an infection. The other infection site was BSI predominantly occurs with a frequency of 8.3 times in SICU than the other surgical wards. This is because; patients in the SICU were critically ill and patients who cannot cough or gag very well are most likely to inhale colonized microorganisms into their lungs.

Some respiratory procedures can keep patients from gagging or coughing. Patients who are sedated or who lose consciousness may also be unable to cough or gag. Therefore, the inhaled microorganism grows in the lungs and causes an infection that can lead to blood stream infection. Moreover, many interventions measures which were risk factors for BSI frequently done in this ward like use of invasive-devices (e.g. venous catheterization, respiratory intubation, urinary or nasogastric tubes); mechanical ventilation; suctioning of material from the throat and mouth; the utilization of drugs such as sedatives; or the influence of surgical procedures. The distribution of nosocomial infection in different site are in agreement with previous studies done in Latvia by Dumpis et al. [19], in Turkey by Durmaz et al. [4], in Taiwan by Wu et al. [20], in Tunisia by Dridi et al. [16] and Cuba by Izquierdo-Cubas et al. [21]. The common interventions done in the hospitals are mechanical ventilation, urinary catheterization, surgical procedures and insertion of central venous lines and not surprisingly, these are responsible for the nosocomial infections. Such invasive procedures are routinely

done in the surgical wards and these wards are becoming reservoirs of multiple drug resistance bacteria. In the present study, these interventions have significant relationship with nosocomial infection. They can also introduce infectious agents to the sites where instruments are placed. This can be favoured for bacterial colonization, which if left unchecked, can become full-blown infections. Considering that these determinants can be altered, efforts to reduce nosocomial infection should be directed along this line. These are in line with other studies in Turkey [22], Kuwait [23], India [12] and Latvia [19]. In order to alleviate this problem, interventions like urinary catheters should only be used when required, should be inserted under aseptic conditions and cleaned daily, should not be left in place for a long time, closed drainage systems should be used when possible and suprapubic catheters should be used in selected cases. Hand washing, a simple but proven practice that reduces nosocomial infection spread should not be forgotten. Among the total bacteria isolated gram-negative bacteria (65.8%) were the predominant isolates while gram-positive bacteria constitute for only 34.2%. Similar findings have been observed in Algeria [24], Tunisia [25] and France [26].

Data presented in this study indicate that most frequent bacterial isolates from surgical site infections were *S. aureus*, *K. pneumoniae* and *P. aeruginosa*. Similar finding have been seen in Turkey [27]. Among urinary tract nosocomial infections *E. coli* is the most frequent bacterial isolate. This finding is also similar with other finding in different publications, Taiwan [12], India [28], Turkey [22], Poland [29], USA [30] and Brazil [31]. *S. aureus* and Coagulase negative staphylococci were the predominant organism in blood stream nosocomial infections. Similar findings have been observed in Taiwan [20].

Currently many micro organisms have become resistant to different antimicrobial agents and in some cases to nearly all agents. Resistance to antimicrobial agents is a problem in health care facilities, but in hospitals, transmission of bacteria is amplified because of the highly susceptible population [1]. The antibiotic sensitivity of our study confirmed the alarming percentage of resistance exhibited by pathogens to the common antibiotics in use. In this particular study, both Gram-positive and Gram-negative bacterial isolates showed high resistance level to ampicillin and chloramphenicol. The result has also indicated that Gram-positive and Gram-negative bacteria isolated from nosocomial infections had low resistance rates (<60%) to ciprofloxacin. Ciprofloxacin and norfloxacin were relatively effective antibiotics for the treatment of pathogens which are responsible to cause nosocomial infections. This is perhaps so because these agents are not commonly used and newly introduced. However, the uses of drugs like norfloxacin are limited in practice because of their high cost. Due to this fact, they showed low level of resistance. According to this study, it appears that the clinician is left with very few choices of drugs for the treatment of nosocomial infections.

In general, rates of resistance to all antibiotics tested for gram negatives were low as compared to gram positives bacterial isolates. However, the present study showed a high prevalence of resistance to the commonly prescribed antimicrobial agents. This may be because of the intense use of

antimicrobial agent in the hospital, easy availability and indiscriminate use of these drugs outside the hospitals, and many antibiotics are available over the counter for self-medication. These problems, coupled with the increase chance of cross infection among inpatients, are known to account for circulating resistance strains. Based on these study the empirical treatment to nosocomial infections provoke drug resistance, therefore treatment should be based on the result of culture and sensitivity. In order to achieve this, the capacity of microbiology laboratory should be strengthened with trained manpower, budget and necessary laboratory equipments. Also, there is a need for a continuous surveillance for resistant bacteria to provide the basis of alternative treatment.

Digestion of this 2.71 kb plasmid with *HindIII* and *EcoRI* resulted in too many and too closely positioned bands to compare restriction patterns properly from one gel to another. All plasmids were therefore digested with *HindIII* and *EcoRI*. With this enzyme, we found 5 different restriction patterns of the 2.71 kb plasmid, some patterns being dominant in certain areas of the world. This indicated that RFLP analysis may be used in epidemiological investigations. The results give evidence to suggest that the virulence plasmid is widespread among some pathogenic strains over the world and has been so for a long time. It is noteworthy that all virulence plasmids from *A. baumannii* strains had identical restriction patterns, indicating a clonal spread in Al Jouf region, KSA.

Minor differences in restriction patterns within the 2.71 kb plasmid were previously reported by Wiik et al., [32] who had studied two plasmids. This plasmid has never been detected alone, only together with a pJM 1-like or pJM 1-derived plasmid. The restriction pattern of this plasmid together with pJM1 is shown in Figs. 2 and 3. The pattern of the 84 k plasmid is thus obtained by subtracting pattern 1 of the pJM1-like plasmid from the restriction pattern of both plasmids together. At present, it is not known whether this plasmid can exist alone, nor do we know anything about the properties it is coding. However, the authors did not show a plasmid profile for this strain, so we suggest that it may in fact have carried plasmids, almost identical to the strain in polymorphism of restriction patterns was not only detected among plasmids but also among other plasmid groups. This suggests that REFP studies may be a general method, applicable for epidemiological studies of pathogenic bacteria.

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