Antimicrobial Resistance of *Acinetobacter* spp. Isolated from Pus Specimens from AL-Shifa Hospital, Gaza, Palestine.

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الملخص

مقاومة المضادات لعزلات الاسينيتوباكتر من عينات الصديد من مستشفى الشفاء بغزة، فلسطين

تعتبر بكتيريا الاسينيتوباكتر مسئولة عن عدد متزايد من الالتهابات الانتهازية في المستشفيات. واكتسبت شهرة واسعة من خلال قدرتها على مقاومة المضادات الحيوية. تعتبر دراسات مقاومة المضادات الحيوية ضرورة ملحة في كل بلدان العالم لمساعدة الأطباء المحليين في وصف العلاجات النظرية. تم في هذه الدراسة جمع عينات الصديد على مسحات أو بواسطة المحاقن من مرضى من مستشفى الشفاء بغزة. العينات زرعت على الأوساط البكتيرية (اجار الدم واجار الماكونكي). تم افتراض العزلات التي امتلكت صفة عدم تخمير الجلوكوز وسالبة فحص الاوكسيديز على أنها اسينيتوباكتر. تم عزل ما مجموعه 152 عزلة من الاسينيتوباكتر وأظهرت نتائج فحص الحساسية مقاومة عالية للعزلات لكل من Cephalosporins (cephalexine, 98%, cefuroxime, 98.2% cefotaxime, 93.2%, ceftazidime, 87.5%, ceftriaxone, (93.3% cefaclor, 97.4%) بينما أظهرت الدراسة نسب أقل من المقاومة ضد aminoglycosides (amikacin, 68.3% and gentamicin, 81.3%). المضاد الأكثر فعالية كما اظهرت النتائج هو doxycycline بأقل نسبة مقاومة (22.1%). نتائج هذه الدراسة يجب أن تعتبر بمثابة إنذار ويجب القيام بخطوات عملية لتقليل مخاطر الاصابة في المستشفيات والبحث عن مضادات بديلة.

كلمات مفتاحية: مقاومة المضادت الحيوية، اسينيتوباكتر.

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ABSTRACT

Acinetobacter spp. is responsible for an increasing number of opportunistic, nosocomial infections. It also gained the reputation of being one of the most efficient pathogens in combating antimicrobials. Antimicrobial resistance studies are world-wide necessity to assist local physicians in prescribing empirical therapy. In this study, pus samples collected on swabs or aspirated in syringes from patients admitted to Al-Shifa hospital in Gaza City were plated on MacConkey agar and Blood agar plates. Oxidase negative, non-glucose fermenter gram negative bacilli were considered as presumptive *Acinetobacter* spp. isolates. A total of 152 strains of *Acinetobacter* spp. were isolated. Antibiogram results to 10 antimicrobials, showed high resistance to most of the commonly used drugs. The isolates showed almost complete resistance to cephalosporins (cephalexine, 98%, cefuroxime, 98.2% cefotaxime, 93.2%, ceftazidime, 87.5%, ceftriaxone, 93.3% cefaclor, 97.4%), while lower rates of resistance were shown against the aminoglycosides (amikacin, 68.3% and gentamicin, 81.3%). The most effective antimicrobial drug as shown by the results of this study was doxycycline with the lowest resistance rate (22.1%). The findings of this study should be considered alarming and actions must be taken to minimize both the risk of nosocomial infections by this pathogen and to search for alternative antimicrobials.

*Key words***:** Antimicrobial resistance, *Acinetobacter* spp.

INTRODUCTION:

Acinetobacter is a saprophytic bacterium found in living organisms and inanimate beings. About 25% of people are healthy carriers. Owing to its scarce virulence, the great majority of infections are produced in the hospital environment, with a greater incidence in patients who are seriously ill and even in a critical state, with central venous lines, vesicle probes, mechanical ventilation, etc. *Acinetobacter* can also be found in the soil, water, pasteurized milk, frozen food, hospital air-conditioning systems, water deposits, dialysis fluids, hospital mattresses, humidifiers, and oxygen systems **(Pedro et al., 2001).**

The emergence and rapid spread of multidrug-resistant isolates causing nosocomial infections are of great concern worldwide. During the last decade, nosocomial infections caused by multidrug-resistant *A. baumannii* have been reported **(Po-Ren et al., 2002).** Almost 25 years ago, researchers

observed acquired resistance of *A. baumannii* to antimicrobial drugs commonly used at that time, among them aminopenicillins, ureidopenicillins, first and second-generation cephalosporins, cephamycins, most aminoglycosides, chloramphenicol, and tetracyclines **(Murray & Moellering, 1979)**. Since then, strains of *A. baumannii* have also gained resistance to newly developed antimicrobial drugs. Although multidrug-resistant (MDR) *A. baumannii* is rarely found in community isolates, it became prevalent in many hospitals **(Zeana et al., 2003).** MDR A. baumannii has recently been established as a leading nosocomial pathogen in several Israeli hospitals **(Simhon et al., 2001 & Melamed et al., 2003).**

Nosocomial *Acinetobacter baumannii* is commonly acquired through cross-transmission because of its propensity to survive in the hospital environment and persistently contaminate fomites **(Borer et al., 2005)**.

Interpreting the significance of isolates from clinical specimens is often difficult, because of the wide distribution of *Acinetobacter* in nature and its ability to colonize healthy or damaged tissue **(Lahiri et al., 2004).** Upto 25% of healthy ambulatory adults exhibit cutaneous colonization and are the most common Gram negative bacilli carried on the skin of hospital personnel **(Mandell et al., 2000).**

In Gaza Strip, there is little or no literature about the epidemiology of *Acinetobacter* spp. or their resistance profile despite the fact that neighboring countries had reported MDR *Acinetobacter*. In this study, isolation of *Acinetobacter* from pus samples collected from Gaza city largest hospital was attempted and their antimicrobial resistance profile was analyzed.

Materials and Methods:

Hospital Setting:

This study was performed at the Al-Shifa Hospital, Gaza, Palestine, which include 489 beds. The collection of pus samples was made over the period from January 2004 to August 2005. Multiple isolates from a single patient were included only if they were recovered from different body sites or recovered from the same site more than 7 days apart.

Specimen Collection, Culturing and Identification:

All samples (a total of no 4184 pus samples) were collected either on sterile swabs or syringes and delivered within one hour **(Anad, 2001)** by the various hospital departments. Samples that exceeded one hour of collection were discarded as inappropriate for culture. Pus samples received in syringes and swabs were cultured onto Blood and MacConkey Agar plates. Plates were incubated at 37 ^{o}C for 24 hours. None lactose fermenting gram negative bacilli were considered as *Acinetobacter* if they were oxidase negative and glucose non-fermenters. API 20 E system **(BioMerieux, Marcy L`Etoil, France)** was used for confirmed identification.

Antimicrobial Susceptibility Testing:

Antimicrobial susceptibility of the isolates was determined by means of the agar diffusion method, according to guidelines established by NCCLS**.** The following antimicrobial agents were used in this study (Piperacillin (100µg), cephalexin (30µg), cefuroxime (30µg), cefotaxime (30µg), ceftazidime (30µg), ceftriaxone (30µg), Amikacin (30µg), gentamicine (10µg), doxycycline (30µg), ciprofloxacin (5µg) and cefaclor (30 µg)). The isolates were grown for four hours in Brain-Heart Infusion Broth (HiMedia) at 37°C. Bacterial inocula were prepared by adjusting the turbidity to a 0.5 McFarland standard. With the use of sterile cotton swab the standardized inoculum was spread onto the Mueller-Hinton agar (HiMedia) and then antimicrobial agents were applied. Plates were incubated at 37°C in ambient air. Organisms were categorized as susceptible, intermediate or resistant to the antimicrobial agent on the basis of guidelines provided by the national committee for clinical laboratory standards **(NCCLS, 2000).**

Data analysis:

Data generated during this research was tabulated and antimicrobial results were expressed as percentage. Chi square (SPSS software) was used to detect statistical differences in the susceptibility pattern of the isolates according to source.

RESULTS:

A total of 152 *Acinetobacter* spp isolates were recovered from the tested sample during the study period. The distribution of the isolates is shown in table 1.

Table 1. Distribution of *Acinetobacter* spp. isolates by department

Department	Frequency	Percent
Burn Unit	17	11.2
Delivery ward	1	0.7
Female clinic	2	1.3
ICU	16	10.5
Internal med	2	1.3
Male clinic	26	17.1
Nursery	27	17.8
Orthopedics	31	20.4
Outpatient	14	9.2
Surgery	16	10.5
Total	**152**	**100.0**

From the above table, it could be noticed that the highest number of isolates was obtained from orthopedic ward (20.4%), followed by nursery ward (17.8%) and male clinic (17.1%0), while the lowest number of isolates was from delivery ward (0.7%) followed by female clinic (1.3%).

Table 2. In vitro susceptibility of *Acinetobacter* spp. isolates to commonly used antimicrobial agents.

Antimicrobials	% Susceptibility		
	S	R	I
piperacillin (N=152)	3.9	94.7	1.3
cephalexin (N=152)	1.3	98.7	0
cefuroxime (N=56)	1.8	98.2	0
cefotaxime (N=146)	3.4	93.2	3.4
ceftazidime (N=144)	3.5	87.5	9.0
ceftriaxone (N=30)	0	93.3	6.7
amikacin (N=145)	14.5	68.3	12.2
gentamicine (N=150)	12.7	81.3	6
doxycycline (N=140)	64.3	22.1	13.6
ciprofloxacin (N=142)	19.7	69.7	10.6
cefaclor (N=151)	1.3	97.4	1.3

S= Susceptible, R= Resistant, I= Intermediate.

Very high percentage of resistance exhibited by the isolates against a variety of antibiotics; cephalexin (98.7%), cefuroxime (98.2%), cefaclor (97.4%), piperacillin (94.7%), ceftriaxone (93.3%), cefotaxime (93.2%), ceftazidime (87.5%). These listed antimicrobials constitute one of the most important groups in treating *Acinetobacter* infection but seem as shown by the results to be overcome by the majority of the isolates.

Also from table 2, doxycycline exhibited the highest activity against the isolates in comparison to other antimicrobials (64.3% of strains susceptible), followed by ciprofloxacin (19.7%) and amikacin (14.5%).

Table 3. Distribution of tetracycline resistance among *Acinetobacter* isolated from various hospital departments

Department	S	R	I
Burn Unit	66.7%	13.3%	20.0%
Delivery ward	100.0%	0.0%	0.0%
Female clinic	50.0%	0.0%	50.0%
ICU	56.3%	37.5%	6.3%
Internal med	50.0%	50.0%	0.0%
Male clinic	72.0%	16.0%	12.0%
Nursery	77.3%	13.6%	9.1%
Outpatient	41.7%	25.0%	33.3%
Surgery	66.7%	20.0%	13.3%
Orthopedics	60.0%	30.0%	10.0%
Total	64.3%	22.1%	13.6%

S= Susceptible, R= Resistant, I= Intermediate

There was no statistically significant difference among the isolates with regard to their resistance to tetracycline according to the source of isolation. In an attempt to detect differences in their susceptibility pattern, clinical isolates were classified according to whether the source is from inpatients or outpatients (table 4). Higher percentages of susceptibility were observed for both ciprofloxacin and Amikacin in outpatient than in inpatient isolates. These differences were not statistically significant.

Table 4. Ciprofloxacin and Amikacin susceptibility in both in and outpatients

Department	Ciprofloxacin			Amikacin		
	S	R	I	S	R	I
Inpatients	18.6%	69.8%	11.6%	13.7%	71.0%	15.3%
Outpatients	30.8%	69.2%	0.0%	21.4%	42.9%	35.7%
Total	19.7%	69.7%	10.6%	14.5%	68.3%	17.2%

S= Susceptible, R= Resistant, I= Intermediate

DISCUSSION:

Acinetobacter infections are serious and difficult to treat owing to their ability to acquire resistance to many of the most commonly used drugs. The aim of this study was to assess the isolation frequency of *Acinetobacter* spp. from pus samples and to study the antibiogram of those isolates. Studies on *Acinetobacter* in various countries **(Villers et al., 1998)** have shown variation in the isolation rates from pus samples (11.7-27.5%) (table 5) . In this study, the lowest rate of *Acinetobacter* from positive pus samples was observed (6.2%). This low rate may not reflect the true *Acinetobacter* spp. infection rates as it was shown by many investigators that this organism is usually mistaken when conventional processes of identification are used **(Lahiri et al., 2004).**

Table 5. Percentage of *Acinetobacter* spp. isolated from pus samples in different countries.

Country	Year of study	*Acinetobacter* % from pus
USA	1977	21.5
France	1991	27.5
Belgium	1991	22.3
Germany	1993	16.4
USA	2000	11.7
Present study		6.2

Large amount of literature was generated all over the world concerning the increasing resistance of *Acinetobacter* spp. and in many of the reviewed literature, the results greatly varied. These variations could be attributed to many factors; one of the most important factors appears to be the variations in treatment protocols implemented by different hospitals. Therefore, it would be impractical for a researcher to compare and interpret the result of his work with others from different localities unless similar conditions of antimicrobial treatment protocols exist. One logical comparison would be the the percentage of resistance to each of the locally used antimicrobial.

Aminoglycosides resistance among the isolated strains was high (Gentamicin, 81.3% and Amikacin 68.3%) and similar results were obtained **(Echeverria et al., 1997).** But much lower resistance to Amikacin was observed in Spain (Ruiz et al., 1999) and in "Israel" **(Simhon et al., 2001).** In a local study conducted to evaluate the overall resistance rates in the most commonly isolated pathogens in Gaza strip **(El-Astal, 2004),** Amikacin was shown to be one of the most effective against gram negative isolates. The fluoroquinolone, ciprofloxacin showed about 30% susceptibility. Similar

results were obtained in Slovakia (Hostacka & Klokocnikova, 2002). The sensitivity of *Acinetobacter* to ciprofloxacin dropped dramatically over the years in "Israel" **(Simhon et al., 2001)**. The United States also experienced great reduction of susceptibility of ciprofloxacin against both *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in both ICU and Non-ICU patients **(Karlowsky et al., 2003).**

Cephalosporins are among the antimicrobials that showed the lowest activity against *Acinetobacter* as shown in table 2. The resistance rates exceeded 90% except for the antibiotic ceftazidime. These results are in agreement with other investigators **(Simhon et al., 2001).**

Amikacin resistance in isolates from inpatients was much higher than those from outpatients while there was no difference in that matter for ciprofloxacin. This could be attributed to the dosage form and the availability of both agents for consumers. Amikacin is only available as injection, and its use is restricted to hospitals, while ciprofloxacin is available in capsule form and is widely prescribed for both in and out-patients.

CONCLUSIONS:

The results of this work indicated high antimicrobial resistance of *Acinetobacter* spp., posing a serious threat to hospitalized patients. A strict attention to maintain and control of the environment and of the antimicrobial use, appears the measures most likely to control the spread of this organism in hospitals. Regular monitoring of the antibiogram of hospital pathogen is also recommended to keep physician updated on the proper empirical treatment of such rapidly evolving resistant pathogens

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