

Mat Soc Med. 2012 Sep; 24(3): 194-197

Received: 11 June 2012

Accepted: 15 August 2012

CONFLICT OF INTEREST: none declared.

© AVICENA 2012

doi: 10.5455/msm.2012.24.194-197

Incidence and Resistotyping Profiles of *Bacillus subtilis* Isolated from Azadi Teaching Hospital in Duhok City, Iraq

Najim Abdulla Yassin¹, Alaa Muyassar Ahmad²

School of Medicine, Faculty of Medical Sciences, Duhok University, Iraq¹

Microbiology Section, Medical-Technique Institute, Duhok City, Iraq²

Corresponding author: Najim Abdulla Yassin, MD. School of Medicine, Medical Faculties, Duhok University, Kurdistan region, Iraq, E.mail: najim56@yahoo.com

ORIGINAL PAPER

ABSTRACT

Background: *Bacillus subtilis* are opportunistic, spore forming bacteria, common soil inhabitants. A resistant spore allows bacteria to endure extreme conditions of heat and desiccation in the environments promotes their survival in many instances, even in environments like hospitals.

Objectives: This paper purposes to find out the incidence of *Bacillus subtilis* from various sources at Azadi Teaching Hospital in Duhok city, Iraq. The susceptibility test and resistotyping (antibiotypes) profile of isolates were also studied. **Methods:** During a period of eight months between Januarys to April, 2011, a total of 128 samples were collected from various sources and locations at Azadi Teaching Hospital in Duhok city. A sterile cotton swabs were used to collect the samples and analyzed by plating on Blood agar, Chocolate agar and MacConkey agar followed by the identification of the isolates based on their cultural characteristics and their reactions in standard biochemical tests. All the isolates were tested for antimicrobial susceptibility by the disk diffusion technique according to the Clinical and Laboratory Standards Institute guidelines on Muller Hinton Agar. **Results:** Out of the 128 collected samples, 84 samples yielded bacterial growth, of them 31(24.2%) were *Bacillus subtilis*. Moreover, other bacterial groups were also isolated and identified. The results showed that the occurrence of *Bacillus subtilis* was higher than the other groups of bacteria. The susceptibility test of *Bacillus subtilis* isolates; the organism exhibited high susceptibility rate to gentamicin (96.7%) and ciprofloxacin (93.5%) While, cefotaxime (19.3%) and ampicillin (16.2%) demonstrated the lowest percentage of susceptibility rate. Resistotyping (antibiotypes) profiles of *Bacillus subtilis* isolates were determined. Out of 31 isolates, 22 of them were multiple resistant and belonged to 3 resistotype patterns; resistotype 1 was predominant among isolates. **Conclusion:** This study shows that there is an increased rate of incidence of *Bacillus subtilis* in hospital environments in study area and some of these isolates were multi-drug resistant and showed different resistotyping profiles.

Key words: *Bacillus subtilis*, Antibiotics, Antibiotypes, Hospital environments.

1. INTRODUCTION

Bacillus subtilis is aerobic, endospore-forming, gram-positive bacteria, opportunistic pathogen and the virulence characteristics of the microorganism are low (1). They are common soil inhabitants and may frequently contaminate foods and widely distributed in hospital environments. The ability of some bacteria to form resistant spores allows it to endure extreme conditions of heat and desiccation in the environment promotes their survival in many instances like hospitals making problems for cleaning and disinfection (2).

Airborne spread has been linked to the development of a cluster of symptoms, particularly in immunocompromised patients, that include eye and sinus irritation, sore throat, headache, fatigue, and dizziness (3). Cases of nosocomial

bacteremia have reported in patients with underlying diseases such as cancer and hematological disorders (4). Moreover, *B. subtilis* does produce an extracellular toxin known as subtilisin, a proteinaceous compound, is capable of causing allergic and hypersensitivity reactions in individuals who are repeatedly exposed to it (5).

Member of this genus are also a well-known food-poisoning organism producing diarrheal enterotoxins. Subsequently, ingestion of contaminated food may be a risk of setting outbreak case (6). Report documented that *Bacillus* isolates belonging to the *B. subtilis* group was cytotoxic (7). It is a real need in monitoring potential spreading of *Bacillus* in hospitals environments to understand the distribution in different outbreak cases. *The aim of this study*

Bacteriological profiles	Number of isolates								No. (%)
	Interior Environment (45)*	Surgical ward (25)*	ENT Unit (14)*	Delivery ward (3)*	Laboratory dept (9)*	Pharmacy dept (9)*	Meeting room (7)*	Kitchen room (16)*	
<i>Bacillus subtilis</i>	10	-	-	-	4	7	5	5	31 (24.2)
<i>Staphylococcus aureus</i>	10	2	3	-	1	-	1	3	20 (15.6)
<i>Staphylococcus epidermidis</i>	5	3	1	-	2	1	1	-	13 (10.1)
<i>Klebsiella pneumoniae</i>	5	-	2	-	1	-	-	-	8 (6.2)
<i>E. coli</i>	2	-	-	-	-	-	-	-	2 (1.5)
Other <i>Bacillus</i> spp	8	-	1	-	-	1	-	-	10 (7.8)
No growth	5	20	7	3	1	-	-	8	44 (34.3)
Total									128 (100)

Table 1. Frequency and percentage of *Bacillus subtilis* and other bacterial groups * Number of collected samples

was based on find out incidence of *Bacillus subtilis* from various sources at Azadi teaching hospital in Duhok city, Iraq. Another aims were evaluation of susceptibility test of isolates against common clinical prescribed antibiotics by our doctors together with establishment of resistotyping profile in order to detect possibility of relatedness among isolates from different sources.

2. MATERIALS AND METHODS

Samples collection: The samples were collected from various sources distributed between interior environments (floors, indoors and walls), ENT unit, surgical ward, delivery ward, laboratory and pharmacy section, meeting hall and kitchen room at Azadi Teaching Hospital in Duhok city, Iraq, from January to April 2011. The samples were collected aseptically used sterile cotton wool swab and being routinely processed by the department of microbiology at medical techniques institute in Duhok city.

Culture media and tests: Several media and tests were used for the isolation, identification and testing the susceptibility of the isolates for common used antibiotics. The media used are Blood agar (with 5-7% defibrinized blood), MacConkey agar, chocolate agar, nutrient agar, Mannitol salt agar, Simmons citrate agar, kligler iron agar (KIA), Mueller-Hinton agar, indole production, motility test (SIM), methyl red-voges proskauer broth, 6.5% NaCL nutrient broth, starch solution, coagulase, catalase, urease, oxidase tests were used for the identification. All of the above media and reagents were obtained from (Difco, USA). The media were prepared according to manufacturer's instructions and sterilized by autoclaving at 121°C for 20 min. The plates were incubated at 37°C for 18-24 h in an incubator. The plates were observed in next day but extended to 48 h if there was no bacterial growth. Isolated colonies were subjected to gram staining technique and biochemical tests for identification as follow:

For Identification of *Bacillus subtilis* isolates: mor-

phological characteristics (no effects on blood agar), gram stain, motility test, starch hydrolysis, voges-proskauer test, citrate utilization and growth in 6, 5% NaCl nutrient broth were performed (8).

For Identification of other groups of bacteria:

Gram-negative bacteria: morphological characteristics, grams stain and motility test were performed. To check the growth pattern MacConkey agar was used. For biochemical characteristics, sugar fermentation, IMVIC test, KIA, urease and nitrate test were performed (8).

Gram-positive bacteria: morphological characteristics and gram stain were performed. To check the growth pattern Blood agar (with 5-7% defibrinized blood), MacConkey agar, chocolate agar, nutrient agar, Mannitol salt agar were used. For biochemical characteristics, sugar fermentation, coagulase, catalase, oxidase test and novobiocin disc (30mg) were performed (8).

Antibiotic susceptibility tests: Antibiotic susceptibility tests were carried out on isolated and identified colonies of *Bacillus subtilis* isolates using commercially prepared antibiotic sensitivity disc (Oxoid, England) using modified Kirby-Bauer method according to CLSI guidelines, using Mueller-Hinton agar standard media. The inhibition zone standards for antimicrobial susceptibility were considered from tables for interpretative zone diameters of Clinical and Laboratory Standards Institute (CLSI) (9). Antibiotics used were amoxiclav (10mg), cephalexin (30mg), gentamicin (10mg), ciprofloxacin (100mg), Cefotaxime (30mg), nitrofurantoin (10mg), amikacin (10mg) and ampicillin (30mg).

In this study resistotyping profile were carried out, briefly: the results of antibiotic susceptibility test against eight different selected antibiotics as described were further characterized by means of typing procedure. A certain patterns of susceptibility profile among isolates of *Bacillus subtilis* were assigned as a resistotype (antibiotype) type (10).

3. RESULTS

A total of 128 samples were collected from various sources at Azadi Teaching Hospital, all samples were directly transferred to the microbiology laboratory and cultured to the appropriate media (as described in materials and methods).

Antibiotics	Symbol	Disc potency (mg)	Susceptibility rate %
Gentamicin	CN	10	96.7
Ciprofloxacin	CIP	5	93.5
Nitrofurantoin	F	10	87.0
Amoxiclav	AMV	30	80.6
Cephalexin	CL	30	25.8
Cefotaxime	CTX	30	19.3
Ampicillin	AM	30	16.2

Table 2. Susceptibility test of *Bacillus subtilis* isolates

Table 1 shows the frequency and percentage of *Bacillus subtilis* and other bacterial groups; the most isolated microorganisms were *Bacillus subtilis* 31 isolates (47.7%). Other groups of bacteria also isolated and identified such as *Staphylococcus aureus* 20(15.6%), *Staphylococcus epidermidis* 13(10.1%), *Klebsiella pneumonia* 8(6.2%), *Escherichia coli* 2 (1.5%), 10(7.8%) other *Bacillus* spp.

Table 2 shows susceptibility test of isolates of *Bacillus subtilis* against common clinical antibiotics, the organism exhibited highly susceptibility rate to gentamicin (96.7%) and ciprofloxacin (93.5%), followed by ciprofloxacin (93.5%), nitrofurantoin (87%) and amoxiclav (80.6%). While cefotaxime (19.3%) and ampicillin(16.2%) showed the lowest

lected from various sources at Azadi Teaching Hospital; 84 samples yielded bacterial growth, of them 31(24.2%) were *Bacillus subtilis*. From interior environments of hospital (floors, indoors, windows and walls) 45 samples were taken 10 samples showed positive growth of *B. subtilis*. Reviews of *Bacillus* infections from several major hospitals suggest that *B. subtilis* is an organism with low virulence. For example, Idhe and Armstrong (1973) reported that *Bacillus* infections were encountered only twelve times over a 6-1/2 year period and species identification of these *Bacillus* infections was not made (11). In another hospital study over a 6-yr period, only two of the 24 cases of bacteremia caused by *Bacillus* (of a total of 1,038 cases) were due to *B. subtilis*. Many of these patients were immunocompromised or had long term indwelling foreign bodies such as a catheter (1). In addition; another study showed that 3% of cases of UTI were caused by *B. subtilis* (12). Because of the pathogenic potential of *B. subtilis* is generally described as low or absent; therefore, data on the general importance of infections due to *B. subtilis* are incomplete, since it is a general practice of most microbiological laboratories to discard these strains or to report them as contaminants.

Our finding demonstrated that the distribution of number of positive growth of *B. subtilis* over number of collected samples from laboratory section, pharmacy section, meeting room, and kitchen room were (4/9), (7/9), (5/7), and (5/16), respectively (Table 1). On other hand, no yields of growth of *B. subtilis* were observed in samples collected from surgical and delivery ward, and ENT unit. The picture however different in a study concerned nosocomial infections, reported one case of throat swab in ENT unit was due to *B. subtilis* (13).

Concerning susceptibility test of *B. subtilis* to antibiotics; in the literature, only a few cases of infections due to *B. subtilis* were reported described the isolation of antibiotic-resistant strains (14, 15, 16). Our results showed the susceptibility rate of *B. subtilis* to gentamicin, ciprofloxacin, nitrofurantoin, and amoxiclav were (96.7%), (93.5%), (87%), and (80.6%) respectively. On other hand, cefotaxime (19.3%) and ampicillin (16.2%) exhibited the lowest percentage of susceptibility rate. This is already in agreement with a study who found that strains of *B. subtilis* isolated from nosocomial infection

manifested high susceptibility to gentamicin (100%) and high resistance to ampicillin (100%) (13). Similar findings reported in another study; resistant rate to gentamicine (8.3%), ciprofloxacin (15%), ampicillin(56%), cephalexin (80%) and cefotaxime (83%) (17). This is already observed in another works (12, 18). Furthermore, one study conducted in Turkey found that the *B. subtilis* produced penicillinase enzyme (19). In contrast to our finding, other study issued that strain of *B. subtilis* from septicemia case, was too sensitive to penicillin, moreover, penicillin was suggested in the treatment of *B. subtilis* infections (4).

Antibiotyping (resistotyping) is a phenotypic method that consists of testing bacterial strains against a set of arbitrarily chosen antibiotics, whereby, a resistance pattern that is characteristic of a strain is generated and, is

Resistotype . No	Resistance profiles	Sources				No. of Isolates (%)
		Interior Environments	Meeting room	Laboratory section	Pharmacy section	
Resistotype I	CL, AM, CTX	3	5	2	6	16 (72.7))
Resistotype II	CL, AM, CTX, AMV	4	-	-	-	4 (18.2)
Resistotype III	CL, AM, CTX, AMV, F	1	-	1	-	2 (9.1)
Total						22

Table 3. Resistotyping profiles of *Bacillus subtilis* isolates

percentage of susceptibility.

Table 3 shows determination of the resistotyping profiles of *Bacillus subtilis* isolates to commonly selected antibiotics. It was found that out of 31 *Bacillus subtilis* isolates, 22 were multiple resistant, i.e. resistant to more than one antibiotic. The obtained results of resistotyping profile (antibiotyping) revealed that 3 distinct resistotyping patterns were found.

4. DISCUSSION

Bacillus subtilis is widely distributed and ubiquitous throughout the environments, particularly in soil and air. It has been shown a capacity to grow over a wide range of temperatures including that of the human body and can temporarily inhabit the skin and gastrointestinal tract of humans (2). Our study investigated 128 samples col-

believed to describe the isolates for epidemiological purposes (20). Obtained results of present study revealed that the 22 *Bacillus subtilis* isolates were multiple resistant and belonged to 3 distinct resistotyping profile (each resistotype involved those strains with identical resistance profile); the predominant one was resistotype I. Moreover, resistotype I included 16 (72.7%) isolates compared with resistotype II and III which included 4(18.2%) and 2 (9.1%) isolates, respectively. Furthermore, isolates of *B. subtilis* belonged to resistotype I had much higher frequency rate in samples that were collected from interior environment, meeting room and laboratory and pharmacy section. A study in Italy, also conducted strain typing by antibiogram (21). Moreover, those isolates, from various sites inside hospital, were identical on the basis of disk susceptibility patterns, indicating relatedness among them. In general, the utility of this simple typing system provide discriminatory between strains and able to determine relatedness among isolates of *B. subtilis* in order to tracing the source of infections in our environment.

In conclusion, this study play a fundamental role in infection control practices and help in the avoidance of nosocomial infections mounting concerns over potential microbial contamination and infection risks in general hospitals.

5. CONCLUSION

This paper purposes to find out the incidence of *Bacillus subtilis* from various sources and locations at Azadi Teaching Hospital in Duhok city, Iraq. The susceptibility test and resistotyping (antibiotypes) profile of isolates were also studied. Samples, using sterile cotton swabs, were collected from various sources and locations and plated on Blood agar, chocolate agar and MacConkey agar. The recovered isolates were identified by routine procedures. Antibiotics susceptibility and resistant profiles to eight selected antibiotics were performed by the disc diffusion method using Mueller-Hinton agar. Out of the 128 samples collected 84 samples were yielded bacterial growth, of them 31(24.2%) were *Bacillus subtilis*. Moreover; other bacterial groups were isolated and identified. The susceptibility test of *Bacillus subtilis* isolates; the organism exhibited high susceptibility to gentamicin (96.7%) and ciprofloxacin (93.5%), while cefotaxime (19.3%) and ampicillin (16.2%) demonstrated the lowest susceptibility rate. resistotyping (antibiotypes) profiles for the isolates of *Bacillus subtilis* were determined. Out of 31 isolates, 22 of them were multiple resistant and belonged to 3 resistotype patterns; resistotype 1 was predominant among isolates. This study shows that there is an increased rate of incidence of *Bacillus subtilis* in hospital environments and some of these isolates were multi-drug resistant and showed different resistotyping profiles.

REFERENCES

- Boer AS, Diderichsen B. On the safety of *Bacillus subtilis* and *B. amyloliquefaciens*: a review. *Appl Microbiol Biotechnol*. 1991; 36: 1-4.
- Garcia-Arribas ML, Rosa MC, Mosso MA. Characterization of the strains of *Bacillus* isolated from orally administered solid drugs. *Pharm Acta Helv*. 1986; 61: 303-307.
- Sativeer SM, Weber DJ, Rutala WA, and Thomann CA. Clinical significance of *Bacillus* species isolated from blood cultures. *South Med J*. 1989; 82(6): 705-709.
- Martha DY, Reinhart JB, and Butler LJ. *Bacillus subtilis* septicemia treated with penicillin. *The J of Pediatrics*. 1949; 35: 237-239.
- Gill DM. Bacterial toxins: A table of lethal amounts. *Microbiol Rev*. 1982; 46: 86-94.
- Oguntoyinbo FA, Sanni AI. Determination of Toxigenic Potentials of *Bacillus* Strains Isolated from Okpehe, a Nigerian Fermented Condiment. *World J of Micro and Biotech*. 2007; 23: 65-70.
- Østensvik ØC, Heidenreich B, O'Sullivan K, Granum PE. Cytotoxic *Bacillus* spp. belonging to the *B. cereus* and *B. subtilis* groups in Norwegian surface waters. *J of Applied Microbiology*. 2004; 96: 987-993.
- Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. *Color Atlas and Textbook of Diagnostic Microbiology*. Philadelphia, Washington: JB: Lippincott; 1992.
- Clinical and Laboratory Standard Institute. Performance standards for antimicrobial susceptibility testing. M100-S16. Wayne, PA: CLSI. 2006.
- Hawkey PM, Lewis DA. *Medical bacteriology a practical approach*. Oxford, England: IPL: Eynsham; 1989: 257-258.
- Ihde DC, Armstrong D. Clinical spectrum of infection due to *Bacillus* species. *Amer J Med*. 1973; 55: 839-845.
- Nadia G, Talat Y, Mujahi, Sumai A. Isolation, Identification and Antibiotic resistance profile of Indigenous of bacterial isolates from urinary tract infection patients. *Pakistan J of Biological Sciences*. 2004; 7: 2051-2054.
- Bienvenido D, Alora MD, Corazon D. Nosocomial Infection: Clinical Features of Two Hundred Forty Three (243) Cases in the Santo Tomas University Hospital. *J Microbiol Infect Dis*. 1983; 12(1): 31-39.
- Velasco E, Sousa M, Tabak D, Bouzas LF. *Bacillus subtilis* infection in a patient submitted to a bone marrow transplantation. *Rev Paul Med*. 1992; 110: 116-117.
- Willet F, Crunelle V, Roussel M, Furchard A, Saunier P, Courcol RJ. *Bacillus subtilis* as a cause of cholangitis in polycystic kidney and liver disease. *Am J Gastroenterol*. 1996; 91: 1477-1478.
- Richard V, Van der Auwera P, Snoeck R, Daneau D, Meunier F. Nosocomial bacteremia caused by *Bacillus* species. *Eur J Clin Microbiol Infect Dis*. 1988; 7: 783-785.
- Esimone CO, Oleghe PO, Ibezim EC, Okeh CO, Iroha IR. Susceptibility-resistance profile of micro-organisms isolated from herbal medicine products sold in Nigeria. *African Journal of Biotechnology*. 2007; 6(24): 2766-2775.
- Balakrishnan S, John KR, Joerge MR. Antibiotic susceptibility of *Bacillus* spp. Isolated from shrimp (*penaeus monodon*) culture ponds. *Indian J of Marine Sciences*. 2003; 32: 81-84.
- Belma A, Necdet S, Yavuz B. Determination of Some Properties of *Bacillus* Isolated from Soil. *Turk J Biol*. 2002; 26: 41-48.
- Adesida SA, Coker AO, Smith SI. Resistotyping of *Campylobacter jejuni*. *Niger Postgrad Med J*. 2003; 10(4):211-215.
- Marco RO, Gianni P, Pier EV, Piero GC. Recurrent Septicemia in an Immunocompromised Patient Due to Probiotic Strains of *Bacillus subtilis*. *J of Clini Micro*. 1998; 4: 325-326.