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

**Najim Abdulla Yassin [1] , Alaa Muyassar Ahmad [2]**

[1]*School of Medicine, Faculty of Medical Sciences, Duhok University, Iraq*

[2]*Microbiology Section, Medical-Technique Institute, Duhok City, Iraq*

(**Received:** 12 / 10 / 2011 ---- **Accepted:** 11 / 6 / 2012)

## Abstract

*Bacillus subtilis* are opportunistic, spore forming bacteria, common soil inhabitants, and may frequently contaminate foods and widely distributed in hospital environments. 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 cephalexin (25.8%), 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.

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

## Introduction

*Bacillus subtilis* is an 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 foods contaminants 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 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.

## 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 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 broth, motility test (SIM), methyl red-voges proskauer broth, 6.5% NaCL nutrient broth, starch solution,. 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:** morphological 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 (30□g) 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 (10□g), cephalexin (30□g), gentamicin (10□g), ciprofloxacin (100□g), Cefotaxime (30□g), nitrofurantoin (10□g), and ampicillin (30□g).

In this study resistotyping profile were carried out, briefly: the results of antibiotic susceptibility test against seven 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]

## 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).

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 pneumoniae* 8(6.2%), *Escherichia coli* 2 (1.5%) and 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 cephalexin (25.8%), cefotaxime (19.3%) and ampicillin(16.2%) showed the lowest 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.

## 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 collected 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) 45samples were collected 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]

**Table1. Frequency and percentage of *Bacillus subtilis* and other bacterial groups**

| Sources | No. of Samples | Number of isolates | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *Bacillus subtilis* | *S. aureus* | *S .epidermidis* | *K. pneumoniae* | *E. coli* | Other *Bacillus* spp | No growth |
| Interior Environments | 45 | 10 | 10 | 5 | 5 | 2 | 8 | 5 |
| Surgical ward | 25 | - | 2 | 3 | - | _ | - | 20 |
| ENT Unit | 14 | _ | 3 | 1 | 2 | - | 1 | 7 |
| Delivery ward | 3 | - | - | _ | - | - | _ | 3 |
| Laboratory dept | 9 | 4 | 1 | 2 | 1 | - | _ | 1 |
| Pharmacy dept | 9 | 7 | - | 1 | _ | - | 1 | - |
| Meeting room | 7 | 5 | 1 | 1 | - | - | - | - |
| Kitchen room | 16 | 5 | 3 | _ | _ | _ | _ | 8 |
| **Total** | 128 (100%) | 31 (24.2%) | 20 (15.6%) | 13 (10.1%) | 8 (6.2%) | 2 (1.5%) | 10 (7.8%) | 44 (34.3%) |

**Table 2. Susceptibility test of *Bacillus subtilis* isolates**

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

**Table 3. Resistotyping profiles of *Bacillus subtilis* isolates**

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

**AM:** ampicillin (30□g), **AMC**: amoxiclav (10□g), **F**: nitrofurantoin (10□g), **CTX**: cefotaxime (30□g), **CL**: cephalexin (30□g).

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 gentamicin (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 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.

## References:


1. Boer AS, Diderichsen B. On the safety of *Bacillus subtilis* and *B. amyloliquefaciens*: a review. Appl Microbiol Biotechnol.1991; 36:1–4.
2. 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.
3. Sativeer SM, Weber DJ, Rutala WA, and Thomann CA. Clinical significance of Bacillus species isolated from blood cultures. South Med J. 1989; 82(6):705-9.
4. Martha DY, Reinhart JB, and Butler LJ. *Bacillus subtilis* septicemia treated with penicillin. The J of Pediatrics. 1949; 35: 237-239.
5. Gill DM. Bacterial toxins: A table of lethal amounts. Microbiol Rev.1982**;** 46:86-94.
6. Oguntoyinbo F A, Sanni A I. Determination of Toxigenic Potentials of *Bacillus* Strains Isolated from *Okpehe*, a Nigerian Fermented Condiment**.** World J Micro and Biotech. 2007; 23:65-70.
7. 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 Appl Microbiol. 2004; 96: 987-993.
8. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Color Atlas and Textbook of Diagnostic Microbiology. Philadelphia, Washington: JB: Lippincott; 1992.
9. Clinical and Laboratory Standard Institute. Performance standards for antimicrobial susceptibility testing. M100-S16. Wayne, PA: *CLSI*. 2006.
10. Hawkey PM, Lewis DA. Medical bacteriology a practical approach. Oxford, England: IPL: Eynsham; 1989.p.257-258.
11. Ihde DC, Armstrong D. Clinical spectrum of infection due to *Bacillus* species. Amer J Med.1973; 55:839-845.
12. 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 Biol Scie. 2004; 7: 2051-2054.
13. Bienvenido D, Alora MD, and 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.
14. 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.
15. Wallet 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?
16. 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.
17. 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 J Biotechnology. 2007; 6 (24): 2766-2775.
18. Balakrishnan S, John KR, Joerge MR. Antibiotic susceptibility of *Bacillus* spp. Isolated from shrimp (penaeus mondon) culture ponds. Indian J of Marine Sciences. 2003; 32:81-84.
19. Belma A, Necdet S, Yavuz B. Determination of Some Properties of Bacillus Isolated from Soil. Turk J Biol. 2002; 26: 41-48.
20. Adesida SA, Coker AO, Smith SI. Resistotyping of *Campylobacter jejuni*. Niger Postgrad Med J. 2003; 10(4):211-5.
21. Marco RO, Gianni P, Pier EV, Piero GC. Recurrent Septicemia in an Immunocompromised Patient Due to Probiotic Strains of *Bacillus subtilis*. J Clini Micro. 1998; 4: 325–326**.**

# نسبة تواجد مع انماط المقاومة للمضادادت الحياتية لبكتريا *Bacillus subtilis* المعزولة من المستشفى الازادي التعليمي في مدينة دهوك-العراق

**نجم عبداللة ياسين ، الاء ميسر أحمد**

[1] *قسم الاحياء المجهرية ، سكول الطب ، فاكولتي العلوم الطبية ، جامعة دهوك ، دهوك ، العراق*

[2] *معهد الطبي التقني، دهوك ، العراق*

**( تاريخ الاستلام:** 12 / 10 / 2011 ---- **تاريخ القبول:** 11 / 6 / 2012 )

**الملخص**

تعتبر *Bacillus subtilis* بكتريا انتهازية، مكونة للسبورات تستوطن التربة وتلوث المواد الغذائية وواسعة الانتشار في بيئات المستشفيات، تستطيع*Bacillus subtilis* ان تستوطن بشكل مؤقت الجلد والقنوات المعدية–المعوية للانسان وقد تمتد الى امكان اخرى في جسم الانسان. معظم الباحثيين سجلوا حالات العدوى داخل المستشفيات وخصوصا تجرثم الدم بسبب هذا النوع من البكتريا والانواع الاخرى . الهـدف من هذه الدراسـة هو لايجاد نسبة تواجد بكتريا *Bacillus subtilis* في داخل بيئة المستشفى(من الجدران الداخلية والممرات والشبابيك والابواب والمقابض والوحدات والردهات المختلفة والاجهزة والادوات والمستلزمات الطبية ومن داخل غرفة المطبخ وغرفة الطعام للاطباء وقاعة المحاضرات).وتحديد مدى حساسية هذه العزلات لمعظم المضـادات الحياتيـة المتداولة، وكذلك اظهار انماط المقاومة لهذه العـزلات لمعرفة مدى تقارب العزلات. تضـمنت هذه الدراسـة 128 عينة جُمعت من اماكن مختلفة في مستشـفى الازادي التعليمي/مدينة دهوك، في الفترة الزمنية بين كانون الثاني –الايار 2011. وتم جمع العينات بإسـتخدام مسحات قطنية معقمة وأخذ عينات من الجدران الداخلية والممرات والشبابيك والابواب والمقابض والوحدات والردهات المختلفة والاجهزة والادوات والمستلزمات الطبية ومن داخل غرفة المطبخ وغرفة الطعام للاطباء وقاعة المحاضرات وزرعها في الاوساط الزرعية التشخيصية والتحضين لمدة 24–48 ساعة في قسم المختبرات في معهد الفني–الطبي بدهوك، وتم تشخيص العُزلات باجراء الاختبارات التشخيصية الروتينية.تمّ إخضاع العُزلات إلى إختبار فحص الحساسية تجاه 8 انواع من المضـادات الحياتيـة وذلك بإسـتخدام طريقة إنتشـار القرص، وايضـاً إيجاد الانماط المقاومة للعُزلات. من بين 128 عينة، 84 عينة اظهرت زرع بكتيري، مِن ضـمنها 31(24.2%) عُزلة *Bacillus subtilis* و 20 (15.6%) عُزلة *Staphylococcus aureus* و13(10,1%)عزلة *Staphylococcus epidermidis* و 8(6.2%)عزلة *Klebsiella pneumonia*، 2(1.5%) عزلة *E. coli*، 10(7,8%)عزلة انواع اخرى من *Bacillus*، و 44(34.3%) لم يعطي زرعاً بكتيرياً.أوضحت النتائج أن نسبة *Bacillus subtilis* كان الاكثر من بين المجاميع البكتيرية المعزولة من داخل بيئة المستشفى الازادي، والنسبة الاقل للاصـابات البكتيرية كانت *E. coli*. أُجري إختبار الحساسية لِعُزلات *Bacillus subtilis* حيث كانت اكثر حساسية للمضادات الحياتية gentamicin تليها ciprofloxacin و nitrofurantoin و amoxiclav ،بينما اظهرت اقل نسبة حساسية للمضـادات الحياتيةcephalexin تليها , cefotaxime وampicillin. كذلك تمّ إجراء تحليل انماط المُقاومة للمضادات الحياتية حيث ظهرت 3 انماط مقاومة مختلفة، وانّ النمط السـائد كانت نمط المقاومة 1.اظهرت هذه الدراسة زيادة في نسبة وجود *Bacillus subtilis* في بيئة الداخلية للمستشفى واظهرت قسم من هذه العزلات مقاومة متعددة للمضـادات الحياتيـة وكذلك اظـهرت انواع مختلفـة من نمط المقاومة تجاه المضادات الحياتية المستخدمة في هذه الدراسة.