Characteristic of *Bacillus* sp. isolated from BasiBhat

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Abstract: Five *Bacillus*were isolated from BasiBhat, which exhibit autoaggregation, cell surface hydrophobicity, resistance to acidic condition (pH 3 for 90 min) and growing in presence of bile salt (0.3%). 16S rRNA gene analysis showed similarity with *Bacillus cereus, Bacillus axarquiensis, Bacillus tequilensis, and Bacillus subtilis (2-strains).* All of them shows susceptible to antibiotics amikacin, amoxycillin, carbenicillin, cefoperazone, cefpodoxime, ceftriaxone, chloramphenicol, chlorotetracyclin, clarithromycin, clindomycin, cephalexin, cephalothin, ciprofloxacin, erythromycin, gatifloxacin, imipenem, kanamycin, nalidixic acid, neomycin, norfloxacin, ofloxin, piperacillin, rifampicin, roxithromycin, streptomycin, Sparfloxacin, tetracyclin, tobramycin, vancomycin, while resistance to penicillin, flucanazole, nystatin, ketoconazole. Similarly all of them are catalase, V.P., and lipase positive, form H_2S from cystein also hydrolyze starch, glycogen, chitin, xylan, gelatin, and casein.

Key words:
BasiBhat, bacillus, probiotic, autoaggregation,Co-aggregation.

Introduction:

The genus *Bacillus* is phylogenetically and phenotypically very heterogeneous (Claus & Berkeley, 1986), it includes more than 100 species (Euzeby, 2004). *Bacillus* is Gram-positive long rods, belonging to Kingdom: Bacteria, Phylum: Firmicutes, Class: Bacilli, Order: Bacillales,Family: Bacillaceae, Genus: Bacillus.

Some *Bacillus* species, such as *Bacillus subtilis*, *Bacillus licheniformis, Bacillus tequilensis, Bacillus axarquiensis* and *Bacillus pumilus*, produce biosurfactants (Arima*et al.*, 1968; Naruse*et al.*, 1990; Yakimov*et al.*, 1995), that reduce surface and interfacial tension with excellent detergent, emulsifying, foaming and dispersing properties. They are preferred over chemical surfactants due to their biodegradability, reduced toxicity, and effectiveness even at extreme temperatures and pH.Surfactin is a cyclic lipopeptidebiosurfactant, which is used extensively in the textile,

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pharmaceutical, cosmetics industries and bioremediation (Banat *et al.*, 2000).

Probiotic strain of *Bacillus* is*Bacillus cereus* which produces lipase, *Bacillus coagulans* reduce cholesterol and with antioxidant activity (Tannock et al). *B. cereus* competes with other microorganisms such as *Salmonella* and *Campylobacter* in the gut and reduces their load. In food animals such as chickens, rabbits and pigs, harmless strains of *B. cereus* are used as a probioticfeed additive to reduce *Salmonella* in the intestines and cecum. This improves the animals' growth and food safety for those who eat their meat.

Bacillus subtilis can produce antibiotics, like polymyxin, difficidin, subtilin, and mycobacillin (Ara 2007). It can degrade polymers such as protein, starch, and pectin. It also acts as biofungicides for benefiting agricultural crops and antibacterial agents. *B. subtilis* inhabits the gut as a normal commensal. Cultures of it is used as an alternative medicine due to the immunostimulatory effects of its cell content. Thus aids in the treatment of gut and urinary tract diseases such as Rotavirus and *Shigella*. It has been safely used in food applications, including the Japanese fermented soy bean, natto. It contributes to a healthy gut flora and vitamin K_2 intake.

Bacillus tequilensis produces pectinase, which is an enzyme group that hydrolyzes pectic substance by depolymerization and de-esterification. Pectinase is of prime importance for plants in cell-wall extension and softening of tissues during maturation, storage, and application in various types of industries such as production of papers and fibers.

Material and methods:

Isolation & Biochemical Characterization:

The *Bacillus* sp. were isolated from BasiBhaton enriched medium; rice powder: 0.5%, peptone: 0.5%, K_2HPO_4: 0.2%, $MgSO_4$: 0.05%,$FeCl_3$: traces, and agar: 2%. All 5 isolated *Bacillus* sp. grew best on tryptone soy agar (TSA). Unless otherwise stated the tests were carried out in TSA medium at 32^0C. Flagella were stained using the method of Rhodes (1958). Spores were stained according to the Schaeffer-Fulton method with 5 days' culture on TSA medium. Growth at different temperatures (5 to 45^0 C) and pH values (5

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to 10) was determined on TSA medium. The catalase and oxidase production, aerobic nitrate and nitrite reduction, H_2S production from cysteine, methyl red, phenylalanine deaminase, lecithinase production, hydrolysis of starch, casein, tyrosine, Tween 20 and Tween 80, haemolysis and growth on MacConkey agar were all assayed according to Barrow &Feltham (1993). Growth in presence of 100 U lysozyme ml^{-1} and dihydroxyacetone production was tested according to Claus & Berkeley (1986). Antimicrobial susceptibility was tested in TSA medium according to the method of Bauer et al. (1966).

Species Characterization on basis of 16S rRNA:

The 16S rRNAgene of isolated Bacillus sp. were amplified by PCR using, respective primer, and following standard protocols.

Forward primer- Bac 8f (5'AGAGTTTGATCCTGGCTCAG3')

Reverse primer - Univ592r (5'ACCGCGGCKGCTGGC3')

The sequence obtained was compared to reference 16S rRNA gene sequences available in the GenBank.

Aggregation test:

Aggregation was considered positive when bacteria gravitated to the bottom of the tubes, leaving a clear supernatant. The test was examined every 15 min for 2 h.

Bile salts tolerance test:

It was determined by sub culturing isolates aerobically at 37°C for 48 h, on bile salt media like DCA, SS agar, and MacConkey agar.

Acidic pH tolerance test:

Cell suspension was prepared as above and then diluted 1 x 10^{-5} in phosphate buffer at pH 3 and 6. The suspensions were then incubated for 90 min at 37°C. The viability was checked by growing the bacterial suspensions on aerobically at 37°C for 48 h.

Antibiogram test:

The inoculums of isolated bacterium were spread evenly over the entire surface of the plates containing nutrient agar. Subsequently, paper discs containing the antibiotics were placed on the plates and incubated aerobically at 37°C for 24 h.

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Cell surface hydrophobicity test:

It was determined by the method of Rosenberg et al. The strain was harvested after 18h of growth, washed twice and suspended in saline solution to OD of 0.5 at 600 nm. To 3 ml of washed cells, 1 ml of toluene added and mixtures were blended for 90 seconds. The tube was left to stand for 15 min for separation; the OD of the aqueous phase was taken. Hydrophobicity was given as percentage decrease in the OD of the bacterial suspension due to partitioning of cells into the hydrocarbon layer. % hydrophobicity = [(OD600 before mixing - OD600 after mixing) / OD600 before mixing] x100 (Handly et al).

Co-aggregation test:

Reid suggested the ability of probiotic organisms to interact closely with pathogenswould constitute an important host defense mechanism against infection. Suspension of the strain, *E. coli, S.aureus,Proteus vulgaris*, or *S.* typhi was adjusted in phosphate buffer (pH 7) to an OD600 of 0.5. A suspension (0.5 ml) of each pathogen and a similar suspension (0.5 ml) of isolated strain was placed together in a test tube and mixed thoroughly. The OD600 of the bacterial mixture was measured after incubation for 4 h at 37°C. Control tubes contained 1 ml of a suspension of each bacterial species. The % of co-aggregation was given by the equation Percentage of co-aggregation = {[(PC+LC)/2-(P+L)] / (PC+LC)/2} ×100. Where PC and LC represent the optical densities in control tubes containing only pathogen or isolated organism after 4 h of incubation, respectively; P+L represent the optical density of the mixed culture after the same period of incubation.

Result and discussion:

Description of *Bacillus tequilensis*-

The sequence obtained for B-10 isolate was compared to reference 16S rRNA gene sequences available in the GenBank, and found 86% identical with *Bacillus tequilensis* strain N-34. It is Gram-positive, aerobic, rods, as single cell or short chains and motile. The endospores are oval and in centre of non-swollen sporangia. On TSA the colonies are yellowish-colored, smooth and circular. In liquid medium a thin film is formed at the surface whilst the

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rest of the medium is slightly cloudy. The optimum growth is at 35^{0}C, pH 7.2. It is both catalase and oxidase positive and reduces nitrate aerobically. Starch, Tween 20, Tween 80, gelatin, and casein are hydrolysed. Voges–Proskauer test, indole, methyl red, citrate, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase and H_2S from cysteine, are positive. It grows in the presence of lysozyme. Tyrosine and urea are not hydrolyzed. Tryptophan and phenylalanine deaminase, pigment after growth on tyrosine medium, H_2S from sodium thiosulphate, is negative. Acids are produced from the following sugars: glycerol, glucose, D-arabinose, L-xylose, L-arabinose, ribose, D-xylose, galactose, sorbose, fucose, mannose, fructose, mannitol, sorbitol, rhamnose, dulcitol, , lactose, sucrose, cellobiose, raffinose, melibiose, inulin, D-lyxose, xylitol methyl □-D-glucoside, maltose, trehalose, starch, glycogen, D-arabitol, and N-acetylglucosamine. Acids are not produced from erythritol, methyl-□-D-xyloside, inositol, gentiobiose, and gluconate. It is susceptible to amoxycillin, clindomycin, chloramphenicol, erythromycin, imipenem, kanamycin, nalidixic acid, norfloxacin, rifampicin, tetracycline, tobramycin, and vancomycin.

Description of *Bacillus axarquiensis*-

The sequence obtained for B-5 isolate was compared to reference 16S rRNA gene sequences available in the GenBank, and found 78% identical with *Bacillus axarquiensis*strain GSO-172. Thecells of the organism are Gram-positive, aerobic, round-ended rods, occurring singly or in pairs and occasionally in short chains or filaments. They are motile by peritrichous flagella. The endospores are mainly ellipsoidal and lie in subterminal positions in non-swollen sporangia. When grown on TSA the colonies are cream-coloured, slightly irregular in shape and bulge upward. When the medium is supplemented with salt the colonies become mucous. In liquid medium a thin film is formed at the surface whilst the rest of the medium is uniformly cloudy. The bacterium grows within a temperature range 15 to 45^{0}C and pH 5 to 10. It is halotolerant, being capable of growth in salt concentrations up to 12%w/v. Optimum growth is at 32^{0}C, pH 7.2 and 0.5% w/v salts. It is catalase-positive and oxidase negative. It reduces nitrate aerobically. Starch, Tween 20, Tween 80, gelatin, casein and lecithin are

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hydrolysed. Citrate is used as sole carbon and energy source. Haemolysis, Voges–Proskauer test, H_2S from cysteine, O-nitrophenyl□-D-galactopyranoside (ONPG) hydrolysis, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are positive. It grows in the presence of lysozyme and in media without yeast extract. Tyrosine and urea are not hydrolyzed. Tryptophan and phenylalanine deaminase, pigment after growth on tyrosine medium, H_2S from sodium thiosulphate, gas from carbohydrates, indole, methyl red are negative. Acids are produced from the following sugars: glycerol, L-arabinose, ribose, D-xylose, fructose, glucose, mannose, inositol, mannitol, sorbitol, methyl □-Dglucoside, cellobiose, maltose, sucrose, trehalose, raffinose, starch, glycogen, xylitol and gentiobiose. Acids are not produced from erythritol, D-arabinose, L-xylose, methyl □-D-xyloside, galactose, sorbose, rhamnose, dulcitol, N-acetylglucosamine, lactose, melibiose, inulin, D-lyxose, D-tagatose, fucose, D-arabitol, gluconate. It is susceptible to amoxicillin, cephalexin, ceftazidime, chloramphenicol, colistin, erythromycin, kanamycin, nalidixic acid, norfloxacin, rifampicin, and vancomycin.

Description of *Bacillus cereus*-

The sequence obtained for B-7 isolate was compared to reference 16S rRNA gene sequences available in the GenBank, and found 86% identical with *Bacillus cereus.*

Thecells of the organism are Gram-positive, aerobic, perfectly rods, occurring in short chains or filaments. They are motile. The endospores are mainly ellipsoidal and lie in subterminal positions in non-swollen sporangia. When grown on TSA the colonies are brown-coloured, wrinkled dull and raised. In liquid medium a thin film is formed at the surface whilst the rest of the medium is slightly cloudy. The bacterium grows within a temperature range 10 to 40^{0}C and pH 5 to 10. Optimum growth is at 37^{0}C, pH 7.2. It is catalase-positive and oxidase negative. It reduces nitrate aerobically. Starch, Tween 20, Tween 80, gelatin, casein and lecithin are hydrolysed. Haemolysis, Voges–Proskauer test, H_2S from cysteine, O-nitrophenyl□-D-galactopyranoside (ONPG) hydrolysis is positive. It grows in the presence of lysozyme and in media without yeast extract. Tyrosine and urea are not

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hydrolyzed. Tryptophan and phenylalanine deaminase, pigment after growth on tyrosine medium, H_2S from sodium thiosulphate, gas from carbohydrates, indole, methyl red, citrate, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase are negative. Acids are produced from the following sugars: glycerol, glucose, methyl □-D-glucoside, maltose, trehalose, starch, glycogen, N-acetylglucosamine. Acids are not produced from erythritol, D-arabinose, L-xylose, L-arabinose, ribose, D-xylose, fructose, methyl-□-D-xyloside, galactose, sorbose, mannose, inositol, mannitol, sorbitol, rhamnose, dulcitol, , lactose, sucrose, cellobiose, raffinose, melibiose, inulin, D-lyxose, xylitol, gentiobiose, fucose, D-arabitol, and gluconate. It is susceptible to amoxicillin, clindomycin, chloramphenicol, erythromycin, imipenem, kanamycin, nalidixic acid, norfloxacin, rifampicin, tetracycline, tobramycin, and vancomycin.

Description of *Bacillus subtilis* subspecies natto-

The sequence obtained for B-3 isolate was compared to reference 16S rRNA gene sequences available in the GenBank, and found 86% identical with *Bacillus subtilis* subspecies natto. Thecells of the organism are Gram-positive, aerobic, rods, occurring as single cell or chains. They exhibit swarming motility. The endospores are mainly oval and lie in central positions in non-swollen sporangia. When grown on TSA the colonies are cream-coloured, large and spreading. In liquid medium a pellicle is formed at the surface whilst the rest of the medium is slightly cloudy. The bacterium grows within a temperature range 5 to 40^{0}C and pH 5 to 10. Optimum growth is at 35^{0}C, pH 7.2. It is catalase and oxidase positive. It reduces nitrate aerobically. Starch, Tween 20, Tween 80, gelatin, and casein are hydrolysed. Voges–Proskauer test, methyl red, citrate, and H_2S from cysteine, are positive. It grows in the presence of lysozyme and in media without yeast extract. Tyrosine and urea are not hydrolyzed. Tryptophan and phenylalanine deaminase, pigment after growth on tyrosine medium, H_2S from sodium thiosulphate, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase, and indole are negative. Acids are produced from the following sugars: glycerol, glucose, ribose, D- galactose, mannose, fructose, mannitol, sorbitol, lactose, sucrose, raffinose, melibiose, inulin,

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methyl □-D-glucoside, maltose, trehalose, starch, glycogen, methyl-□-D-xyloside, cellobiose, gluconate and N-acetylglucosamine. Acids are not produced from erythritol, inositol, D-lyxose, xylitol, D-arabinose, xylose, L-arabinose, sorbose, rhamnose, fucose, arabitol, gentiobiose, and dulcitol,. It is susceptible to amoxycillin, clindomycin, chloramphenicol, erythromycin, imipenem, kanamycin, nalidixic acid, norfloxacin, rifampicin, tetracycline, tobramycin, trimethoprim, and vancomycin.

Description of *Bacillus subtilis* strain VP-80-

The sequence obtained for B-6 isolate was compared to reference 16S rRNA gene sequences available in the GenBank, and found 86% identical with *Bacillus subtilis* strain VP-80. Thecells of the organism are Gram-positive, aerobic, rods, occurring as single cell or chains. They exhibit swarming motility. The endospores are mainly oval and lie in central positions in non-swollen sporangia. When grown on TSA the colonies are cream-coloured, large and spreading. In liquid medium a pellicle is formed at the surface whilst the rest of the medium is slightly cloudy. The bacterium grows within a temperature range 5 to 40^{0}C and pH 5 to 10. Optimum growth is at 35^{0}C, pH 7.2. It is catalase and oxidase positive. It reduces nitrate aerobically. Starch, Tween 20, Tween 80, gelatin, and casein are hydrolysed. Voges–Proskauer test, methyl red, citrate, and H_2S from cysteine, are positive. It grows in the presence of lysozyme and in media without yeast extract. Tyrosine and urea are not hydrolyzed. Tryptophan and phenylalanine deaminase, pigment after growth on tyrosine medium, H_2S from sodium thiosulphate, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase, and indole are negative. Acids are produced from the following sugars: erythritol, glucose, D-lyxose, xylose, D- galactose, gentiobiose, fructose, mannitol, sorbitol, dulcitol, rhamnose, sucrose, raffinose, melibiose, inulin, methyl □-D-glucoside, maltose, trehalose, starch, glycogen, methyl-□-D-xyloside, gluconate and N-acetylglucosamine. Acids are not produced from glycerol, ribose, inositol, xylitol, D-arabinose, L-arabinose, mannose, sorbose, fucose, lactose, cellobiose, and arabitol. It is susceptible to amoxycillin, clindomycin, chloramphenicol, erythromycin,

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imipenem, kanamycin, nalidixic acid, norfloxacin, tetracycline, tobramycin, trimethoprim, and vancomycin.

Table. 1- Cell surface hydrophobicity & Aggregation time of Bacillus sp.

Bacillus sp.	Cell surface hydrophobicity	Aggregation time
Bacillus cereus	81.2%	56 minutes
Bacillus axarquiensis	78.2%	64 minutes
Bacillus tequilensis	86.3%	61 minutes
Bacillus subtilis (B-3)	82.8%	52 minutes
Bacillus subtilis (B-6)	83.4%	55 minutes

Table. 2-Some characteristics of isolated Bacillus sp.

Characteristic	B.cereus	B.axarquiensis	B.tequilensis	B.subtilis (B-3)	B.subtilis (B-6)
Pigment	Brown	Cream	Yellowish	Cream	Cream
Growth on TSA	Wrinkled, dull, raised	Irregular Bulge upward	Smooth, circular	Large, spreading	Large, spreading
Motility	Swarming	Swarming	Swarming	Swarming	Swarming
Spores	Ellipsoidal, subterminal	Ellipsoidal, subterminal	Oval, central	Oval, central	Oval, central
Catalase	+	+	+	+	+
Oxidase	-	-	+	+	+
Starch	+	+	+	+	+
Glycogen	+	+	+	+	+
Chitin	+	+	+	+	+
Pectine	-	+	+	+	+
Xylan	+	+	+	+	+
Inulin	-	-	+	+	+
Gelatin	+	+	+	+	+
Casein	+	+	+	+	+
Indole	-	-	+	-	-
M R	+	+	+	+	+
V P	+	+	+	+	+
Citrate	-	+	+	+	+
H_2S from Cystein	+	+	+	+	+
Urease	-	-	-	-	-
Lipase	+	+	+	+	+
Lecithinase	+	+	-	-	-
Arginine dihydrolase	-	+	+	-	-
Lysine decarboxylase	-	+	+	-	-
Ornithine decarboxylase	-	+	+	-	-
Phenylalanine deaminase	-	-	-	-	-
Haemolysis	+	-	-	-	-

Table. 3-Carbohydrates utilization as acid formation by isolated Bacillus sp.

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Acid from	B.cereus	B.axarquiensis	B.tequilensis	B.subtilis (B-3)	B.subtilis (B-6)
Glycerol	+	+	+	+	-
Erythretol	-	-	-	-	+
D-Arabinose	-	-	+	-	-
L-Arabinose	-	+	+	-	-
D-Ribose	-	+	+	+	-
Arabitol	-	-	+	-	-
D-Xylose	-	+	+	-	+
D-Lyxose	-	-	+	-	+
Xylitol	-	+	+	-	+
D-Glucose	+	+	+	+	-
D-Fructose	-	+	+	+	+
D-Galactose	-	-	+	+	+
D-Fucose	-	-	+	-	-
D-Sorbose	-	-	+	-	-
Sorbitol	-	+	+	+	+
Dulcitol	-	-	+	+	+
Meso-Inositol	-	+	-	-	-
D-Mannose	-	+	+	+	-
Mannitol	-	+	+	+	-
Gluconate	-	-	-	+	+
Gentibiose	-	+	-	-	+
Rhamnose	-	-	+	-	+
Sucrose	-	+	+	+	+
Maltose	+	+	+	+	+
Trehalose	+	+	+	+	+
Cellubiose	-	+	+	-	+
Lactose	-	-	+	+	-
Melibiose	-	-	+	+	+
Raffinose	-	+	+	+	+
Salicine	-	+	-	-	+
N-Acetyl glucosamine	+	-	+	+	+
□-Methyl-D-xyloside	-	-	-	+	+
□-Methyl-D-glucoside	+	+	+	+	+

Table. 4-Antibiotics sensitivity test for isolated Bacillus sp.

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Antibiotic	Abb	B.cereus	B.axarq-uiensis	B.tequi-lensis	B.subtilis (B-3)	B.subtilis (B-6)
Amikacin	Ak^{10}	2.4	1.2	2.6	1.4	1.8
Amoxycillin	Am^{30}	1.1	1.6	1.3	1.6	1.7
Amphotericin	Ap^{100}	-	-	1.6	-	-
Ampicillin	A^{10}	-	-	2.1	2.3	2.1
Aztreonam	Ao^{30}	-	-	1.2	1.6	1.9
Bacitracin	B^{10}	1.4	1.6	1.9	-	-
Carbenicillin	Cb^{100}	1.3	1.7	1.5	1.8	1.8
Cephalexin	Cp^{30}	-	2.1	-	1.2	1.3
Cefoperazone	Cs^{30}	2.7	2.2	2.6	2.1	2.0
Cefpodoxime	Cep^{10}	1.1	1.2	1.5	2.0	1.8
Ceftazidime	Ca^{30}	-	2.3	-	-	-
Ceftriaxone	Ci^{30}	1.4	1.7	1.6	1.3	1.2
Chloramphenicol	C^{30}	3.0	2.0	3.2	2.6	2.4
Chlorotetracyclin	Ct^{30}	1.2	1.3	1.3	1.9	2.0
Clarithromycin	Cw^{15}	2.3	2.6	2.0	2.1	2.4
Clindomycin	Cd^{10}	4.5	3.8	4.2	3.2	3.3
Cloxacillin	Cx^{30}	-	-	1.6	1.5	1.4
Cephalexin	Cp^{30}	1.2	1.0	-	1.3	1.2
Cephalothin	Ch^{30}	1.0	1.3	1.3	1.5	1.4
Ciprofloxacin	Cf^{10}	2.1	2.2	2.4	2.0	2.4
Colistin	Cl^{25}	-	2.2	-	-	-
Erythromycin	E^{15}	2.2	2.5	2.4	3.0	3.0
Flucanazole	Fu^{25}	-	-	-	-	-
Gatifloxacin	Gf^{10}	3.6	3.8	3.2	3.0	2.2
Imipenem	I^{10}	4.5	4.2	4.1	4.0	3.8
Kanamycin	K^{30}	2.4	2.3	2.2	2.6	2.4
Ketoconazole	Kt^{10}	-	-	-	-	-
Nalidixic acid	Na^{30}	2.2	2.4	2.1	2.6	2.0
Nystatin	Ns^{100}	-	-	-	-	-
Neomycin	N^{30}	1.8	1.9	1.6	1.9	1.4
Norfloxacin	Nx^{10}	2.2	2.7	2.0	2.3	2.8
Penicillin	P^{10}	-	-	-	-	-
Ofloxin	Of^{5}	1.4	1.9	1.8	1.6	1.5
Oxacillin	Ox^{5}	-	1.8	-	-	-
Piperacillin	Pc^{100}	1.5	1.9	1.6	1.2	1.6
Rifampicin	R^{30}	1.1	1.6	1.3	1.6	-
Roxithromycin	Ro^{30}	2.4	2.2	2.3	2.0	2.2
Streptomycin	S^{10}	2.0	2.5	2.1	2.4	2.6
Sparfloxacin	Sc^{5}	2.2	2.6	2.6	2.1	2.4
Tetracyclin	T^{30}	2.1	2.3	2.2	2.8	2.1
Trimethoprim	Tr^{5}	-	-	-	2.5	2.8
Tobramycin	Tb^{30}	2.2	2.6	2.1	2.4	2.5
Vancomycin	Va^{30}	1.9	1.8	1.7	2.8	2.6

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