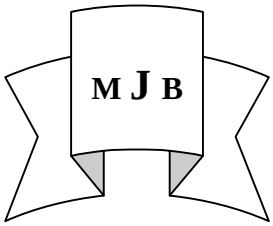
Antimicrobial resistance of bacteria isolated from powdered infant formulas (PIF)

Bushra J. Al-Timimi

College of Medicine, Babylon University.



Abstract

A total of 65 samples of powdered infant formula (PIF) and dried infant cereals were collected at random from different specialties that are available in Hilla city/Iraq, during a 3-month period from October to December 2006. The morphological and physiological characterization of bacterial isolates and antimicrobial susceptibility testing were performed. Bacterial isolates were cultured from 53.8 % of 65 milk substitute infant formulas. The concentration did not exceed a level of 10 CFU/g in any product. The most commonly identified isolates were *Bacillus subtilis* ,*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, ,and *Citrobacter freundii*.One isolate of *Enterobacter sakazaki*, was also detected. Antimicrobial susceptibility testing showed significant resistance rates, particularly to ampicillin, amoxicillin/clavulanic acid, cefotatxime, and gentamicin, but they were highly sensitive to ciprofloxacin and Refampin.

دراسة مقاومة المضادات الحياتية للبكتريا المعزولة من مسحوق حليب الأطفال

الخلاصة

تم جمع 65 عينة مسحوق حليب الأطفال ومستحضرات حبوب الأطفال الجافة من الأسواق المحلية في مدينة الحلة/العراق للفترة من تشرين الأول لغاية كانون الأول 2006.تم دراسة الخصائص المظهرية والفسلجية للعزلات البكتيرية وكذلك دراسة قابلية العزلات على مقاومة عدد من المضادات الحياتية .كانت نسبة عزل السلالات البكتيرية 53.8% من مجموع العينات المدروسة ،وكان عدد الخلايا المعزولة 10 خلية/غرام من كل من العينات المدروسة .كانت أكثر العزلات التي تم الحصول عليها تعود للأنواع البكتيرية *Bacillus subtilis,Staphylococcus aureus,Klebsiella pneumoniae,Enterobacter cloacae,Citrobacter freundii* .كما تم الحصول على عزلة واحدة من *Enterobacter sakasaki*.اظهرت نتائج مقاومة المضادات الحياتية معدلات مقاومة عالية لمضادات الامبسلين والاموكسلين والكلافيولانك والسيفوتاكسيم والجنتامايسين ،*الا ان هذه العزلات كانت حساسة بنسبة كبيرة لمضادي السبروفلاكسين والريفامبين*.

Key words: powdered infant formula (PIF), bacteria, antimicrobial resistance.

Introduction

Feeding with powdered infant formula (PIF) has been epidemiologically implicated in several clinical cases. Infants should be exclusively breast-fed for the first 6 months of life, and those who are not should be provided with a suitable breast-milk substitute. PIF is not a sterile product; to reduce the risk of infection, the reconstitution of powdered formula should be undertaken by caregivers using good hygienic measures and in accordance with the product manufacturer's food safety guidelines (1). Several members of the family *Enterobacteriaceae* have been isolated from powdered infant formula like *E. coli, Klebsiella pneumoniae, Enterobacter clocae, Enterobacter agglomerans*, etc.(2, 3). PIF products have been also shown to contain *Enterobacter sakazakii* and have been epidemiologically linked to several clinical cases (4, 5, 6,7, 8). Although the environ-mental source of *E sakazakii* has not been conclusively established, epidemiological studies implicate dried infant formula as the route of transmission to infants (2, 7, 9). The bacterium has been isolated from powdered infant formula by several investigators (10**,** 11, 12).

The number of bacterial strains capable of causing infections is increasing , and many of them are resistant to one or more of the antibiotics used in therapy and this resistance constitute an increasingly serious threat to the current antimicrobial agents therapy (13, 14). The acquisition of resistance to the antimicrobial agents may be due to chromosomal mutation or plasmids that are capable of transfer from one strain of bacteria to another , even across the species barrier. The aim of this study was to investigate the microbial contamination of some infant formulas present in Hilla city/Iraq which were obtained from various countries and study the susceptibility to selected antimicrobial agents of bacterial strains isolated from these infant formulas.

Materials and methods

Sampling:

A total of 65 samples of powdered infant formula (IF) were collected at random from different specialties that are available in Hilla city/Iraq, during a 3-month period from October to December 2006.

The countries and the corresponding numbers of specimens tested were as follows: Australia, **2**; Belgium, **1**; Brazil, **2**; People's Republic of China, **6;** Thailand, **3**; France, **6**; Germany, **2**; India, **7**; Italy, **3;** Japan, **4**; Kingdom of Saudi Arabia, **5**; Malaysia, **4**; Poland, **2**; Spain, **2;** Tunisia, **3;** Turkey, **6**;

United Arab Emirates, **5.** and Vietnam, 2.The names of the products are available upon request.

Sample analysis:

(**1**) Decontamination. The lid margins of the tins and the spoons used for withdrawal of the samples were flamed with 93% (wt/vol) ethanol. The surfaces of the other packages were disinfected with 70% (wt/vol) ethanol before the samples of the milk powders were withdrawn.

(**2**) Enumeration of the bacterial isolates. Initially, 90, and 9 ml of phosphate buffer solution (pH 7.2) were added slowly at a temperature of 45°C to 10, and 1g, respectively, of milk powder in glass containers, which were swirled by hand until the powder was dissolved. All tests were performed in triplicate. The glass containers were incubated in a water bath at 45°C for 5 min to dissolve the powder. After that,10 ml of the dissolved milk was withdrawn from each flask and added to 90 ml of Nutrient broth (Oxoid,U.K). After overnight incubation, aerobic viable bacteria were isolated by plating of decimal dilutions on the following culture media: nutrient agar, 5 % human blood agar, MacConkey agar, eosin-methylene blue agar, and chocolate blood agar. The cultures were incubated overnight at 36°C. Growth on the agar was subcultured on tryptone soy agar slants and the strains were identified to the level of species using conventional biochemical tests (15,16,17). Additional tests were used to identify *Enterobacter sakazakii:* the production of yellow colonies on nutrient agar (Difco, Detroit.) after 48 h at 25°C**,** the production of extracellular DNase on toluidine blue agar after 6 days at 36°C, and a positive glucosidase reaction after 4 h (2).

Antimicrobial susceptibility tests:

The resistance of the bacterial isolates to antimicrobial agents was determined using disk diffusion method and interpreted according to National committee of clinical laboratory standards-NCCLS-documents (18 ,19).

The following antimicrobial agents were obtained (from Oxoid, U.K) as standard reference disks as known potency for laboratory use: Penicillin (P), Ampicillin (Amp), Amoxicillin (Amx),Cephalothin (CN),Cefotaxime (Ctx), Ceftizoxime (Czx), Amoxicillin /Clavulanic acid (AMC), Gentamycin (Gn), Streptomycin (S), Erythromycin (E), Chloramphenicol (C) , Rifampicin (R), Polymyxin B (PB), Bacitracin (B), Ciprofluxacin (Cp) and Trimethoprim–Sufamethoxazole (SXT).

All these tests were performed on plates of Muller-Hinton agar (Oxoid, U.K). A 0.5

MacFarland suspension was applied to the plates, which were dried in an incubator at 35ºC for 10 minutes. Antimicrobial disks were placed on the agar with sterile forceps. The agar plates were incubated at 35 ºC for 18 hours. The results were recorded by measuring the inhibition zone in millimeters (18).

Results and Discussion

Different bacterial species were detected in the PIF samples tested. Bacterial species were cultured from **53.8 % of 65** milk substitute infant formulas. **71 %** of bacterial species (No.27) were belonged to G+ve bacteria while **28.9 %** of them (No.11) were belonged to G-ve bacteria-namely the members of family Enterobacteriaceae. (**Table-1**). The count of the bacterial cells range between 1-10 cell/ml of the medium. However the concentration did not exceed a level of 10 CFU/g in any product**.**

The results showed that *Bacillus* species were the bacteria most frequently found in PIF samples. The significant microbial contamination of PIF samples with these bacteria is attributed to that *Bacillus* species are found ubiquitously and widely distributed in the soil, dust, air, and water and because they are resistant to environmental destructive factors (20, 21). With the exception of *Bacillus anthracis* and *B. cereus*, the members of the genus *Bacillus* have been frequently considered as non-pathogenic, however, some authors have reported serious human infections associated with *Bacillus* spp. particularly in immunosuppressed patients (22 ,23).other *Bacillus* species are generally perceived as inconsequential and of little clinical significance (24). Due to the endospore-forming ability of members of this genus, these bacteria tolerate adverse conditions better than most bacterial enteropathogens. They occur frequently in hospital foods (25) and domestically prepared foods (9). It should be noted that reconstituted infant milk formula (IMF) were frequently contaminated with acceptably low numbers of *Bacillus* spores (9). Properly reconstituted infant milk formulae (IMF) containing *Bacillus* spp. have not been previously associated with food-borne illness in infants.

Like *Bacillus* spp., Gram- positive cocci can survive in the environment and thus contaminate the PIF. *Staphylococcus epidermidis* was the most frequently isolated species from the PIF products (25.7%). *Staphylococcus aureus* isolates were also isolated from these products (**Table-1**). The contamination of the PIF with this bacteria may pose an important and serious problem because it may generate a severe infections to human.

The presence of Gram- negative rods in these products was expected because of their highly metabolic activity. However only few numbers of Gram- negative rods were recovered from these products (**Table-1**). The isolated species were *Klebsiella pneumoniae, Enterobacter cloacae, Citrobacter freundii,* and *Enterobacter sakazakii,*. If infections due to these organisms occur,it can be useful to include a check of the hygienic precautions which are taken during the preparation and storage of the formula. Milk powders without members of the Enterobacteriaceae might offer extra protection to the newborn if some multiplication does occur in the formula (9).

One of the most striking findings in present study was the isolation of *Enterobacter sakazakii,* the most common bacterial species isolated from PIF products over the world (1, 4, 6, 10**,** 11, 12, 26). Further, there have been many recalls of *E. sakazakii*-contaminated infant formula in the United States. In November 2002, a nationwide recall of more than 1.5 million cans of dry infant formula contaminated with *E. sakazakii* was reported (26).

Although one isolate of *E. sakazakii* was cultured from one sample of formula powders, this might have been due to an unequal distribution in the powder or its presence at such a low concentration that it escaped detection by conventional methods. So large amounts of powdered substitutes for breast milk should be obtained from different sources to be analyzed for the presence of this bacterium.

Related coliforms, which can also cause invasive infections such as *Klebsiella pneumoniae, Enterobacter cloacae,* and *Citrobacter diversus,* have also been isolated from PIF by several investigators (2, 27). There is also evidence from surveillance activities that low-level contamination of PIF with *Salmonella* species has led to cases of disease in infants (28).

Results of this study about resistance of *Bacillus* strains to antimicrobial agents showed that *Bacillus subtilis strains* were sensitive to most of the antimicrobial agents tested **(Table-2)** and these results were similar to those clinical strains (29).

The high proportion of resistant strains of *Bacillus* spp. to ampicillin and erythromycin refers that these antibiotic are not the most suitable antibiotics for these bacteria and this conclusion is similar to the opinion of de la Rosa *et. al*. (30) who isolated these bacteria from non-sterile pharmaceuticals.

Results showed that the strains of *Staph. aureus* isolated in this study were highly resistant to β-lactams, gentamycin, and erythromycin **(Table-2)**, and this in agreement

with several studies of clinical strains (31, 32 , 33), but disagree with studies of medica-ment strains reported by other authors (30) .

Staph. epidermidis had the highest number of mutiresistant strains and these findings are in agreement with those isolated from clinical specimens (34). *Staph. epidermidis* may acts as a reservoir for resistance which can be transferred to *Staph. aureus* .The transfer of resistance among different genera of Gram-positive and Gram-negative bacteria and between *Bacillus* species and Staphylococci has been reported by many authors (35, 36, 37).

The high level of resistance to many antimicrobial agents shown by Gram-negative rods is well known and has been reported with increasing frequency (2, 32, 38,39 , 40).

Klebsiella ,Enterobacter, and *Citrobacter* are of increasing importance , as resistance to newer beta-lactams particularly extended-spectrum beta-lactams may be acquired by mutation in addition to plasmids (32, 41, 42).

Resistance to beta-lactam antibiotics in Gram- negative bacteria can be due to four mechanisms: decreased permeability of the drug into the cell, hydrolysis of the drug by ß-lactamase ,decreased affinity of the target penicillin-binding proteins– PBPs-, or by pump-mediated resistance (42). The major mechanism of resistance in bacteria causing clinically significant infection remains the expression of ß-lactamases, of which there are several classes including plasmid-encoded and chromosomally-encoded enzymes (43).

Klebsiella pneumoniae strains showed multi-resistance to penicillins and most of the other antimicrobial agents tested. in addition to these antibiotics, they showed resistance to extended-spectrum beta-lactams (Ctx and Czx) **(Table-3)**. These results are in agreement with those of PIF (2) and clinical strains reported by several authors (38, 39, 41, 44).

Enterobacter and *citrobacter* strains isolated in the present study also show resistance to β-lactams and other antibiotics studied.These result confirmed by many studies by several investigators (38, 41, 42). The results of this study indicate a high rate of resistance to antimicrobial agents of bacterial strains isolated from PIF products , and this may indicate a widespread antibiotic resistance among bacteria isolated from different sources , including that of anthropological and environmental origin.

References

1. Drudy D, Mullane NR, Quinn T, Wall PG, Fanning S. 2006 . *Enterobacter sakazakii*: an emerging pathogen in powdered infant formula. Clin Infect Dis. 1;42 (7): 996-1002.
2. Carneiro LA, Silva AP, Merquior VL, Queiroz ML. 2003. Antimicrobial

resistance in Gram-negative bacilli isolated from infant formulas. FEMS Microbiol Lett. 1;228 (2): 175-9.

3. Muytjens H. L., ROELOFS-Willemse H., and Jaspar G. H. J.1988. Quality of powdered substitutes for breast milk with regard to members of the family enterobacteriaceae. J. clin. Microbiol., 26 (4):743-746.

4. Simmons BP, Gelfand MS, Haas M, Metts L, Ferguson J. 1989. *Enterobacter sakazakii* infections in neonates associated with intrinsic contamination of a powdered infant formula. Infect Control Hosp Epidemiol. 10:398–401.

5. van Acker J, de Smet F, Muyldermans G, Bougatef A, Naessens A, Lauwers S. Outbreak of necrotizing enterocolitis associated with *Enterobacter sakazakii* in powdered milk formula. J Clin Microbiol 2001; 39:293–7.

6. Himelright L, Harris E, Lorcg V, Anderson M. *Enterobacter sakazakii* infections associated with the use of powdered infant formula—Tennessee. JAMA 2002; 287:2204–5.

7. US Food and Drug Administration. 2005. Health professionals letter on *Enterobacter sakazakii* infections associated with use of powdered (dry) infant formulas in neonatal intensive care units. http:// www.cfsan.fda.gov/˜dms/inf-ltr3.html.

8. Food Safety Authority of Ireland. Pregestimil infant formula recall. Available at: http://www.fsai.ie/alerts/archive/fa20050120.asp. 2006.

9. Rowan, N. J., J. G. Anderson, and A. Anderton. 1997. Bacteriological quality of infant milk formulae examined under a variety of preparation and storage conditions. J. Food Prot. 60:1089–1094.

10. Biering, G., S. Karlsson, N. C. Clark, K. E. Jonsdottir, P. Ludvigsson, and O. Steingrimsson. 1989. Three cases of neonatal meningitis caused by *Enterobacter sakazakii* in powdered milk. J. Clin. Microbiol. 27:2054–2056.

11. Iversen, C., and S. Forsythe. 2004. Isolation of *Enterobacter sakazakii* and other *Enterobacteriaceae* from powdered infant formula milk and related products. Food Microbiol. 21:771–777.

12. Postupa, R., and E. Aldova. 1984. *Enterobacter sakazakii*: a Tween 80 esterasepositive representative of the genus *Enterobacter* isolated from powdered milk specimens. J. Hyg. Epidemiol. Microbiol. Immunol. 28:435–440.

13. Jones, R.N. (2001) Resistance patterns among nosocomial pathogens, trends over the past few years.Chest,119:397S-404S.

14. Verhoef, J. (1994) Worldwide resistance against antibiotics. Int. J. Antimicrob. Agents, 4:141-142.

15. MacFaddin, J.F. (2000) Biochemical tests for identification of medical bacteria. Lippincott Williams & Wilkins. Philadelphia, USA.

16. Cowan, S.T. (1985) Cowan and Steels' manual for identification of medical bacteria. 2nd edn., Cambridge Univ. press. U.K.

17. Claus, D. and Berkeley R.C.W. (1984) Genus *Bacillus*,.1105 –1139, In : Sneath, P.H.A., Mair, N.S., Sharpe, M.E., and Hoid, G.(eds) Bergeys' manual of systematic bacteriology. vol. 2. The Williams & Wilkins Co. Baltimore.

18. National Committee for Clinical Laboratory Standards (NCCLS). 2003.

Performance standards for disk susceptibility tests, 8th ed. Approved standard M2-A8. National Committee for Clinical Laboratory Standards, Wayne, Pa.

19. National Committee for Clinical Laboratory Standards (NCCLS). 2003. Performance standards for antimicrobial susceptibility testing. approved standard M 100-S13. National Committee for Clinical Laboratory Standards, Wayne, Pa.

20. Gordon, R.E., Hayes, W.C., and Pant, C.H. (1973) The genus Bacillus. US department of agriculture, agricultural book No. 427 . Washington, USA .

21. Beattie, S. H., and A. G. Williams. 1999. Detection of toxigenic strains of *Bacillus cereus* and other *Bacillus* spp. with an improved cytotoxicity assay. Lett. Appl. Microbiol. 28:221–225.

22) Cotton, D.J., Gill, V.J., Mashall, D.J., Gress, J. Thaler, M., and Pizzo, P.A. Clinical features and therapeutic intervention in 17 cases of Bacillus bacteremia in an immunosuppressed patient population . J. Clin. Microbiol., 25 : 672 – 674 .

23) Sliman, R., Rehn, S., and Shlaces , D.M. (1986) Serious infections caused by *Bacillus* species. Medicine, 66 : 218-223 .

24) Drobniewski, F. A. 1993. *Bacillus cereus* and related species. Clin. Microbiol. Rev. 6:324–338.

25) Rowan, N. J., and J. G. Anderson. 1998. Growth and enterotoxin production by diarrhoeagenic *Bacillus cereus* in dietary supplements prepared for hospitalized HIV patients. J. Hosp. Infect. 38:139–146.

26) . Gurtler JB, Kornacki JL, Beuchat LR. 2005. *Enterobacter sakazakii:* a coliform of increased concern to infant health. Int J Food Microbiol. 25; 104(1):1-34.

27) Thurm V, Gericke B. Identification of infant food as a vehicle in a nosocomial outbreak of *Citrobacter freundii:* epidemiological subtyping by allozyme, whole-cell protein and antibiotic resistance. J Appl Bacteriol 1994; 76:553–8.

28) Usera MA, Echeita A, Aladuena A, et al. Interregional foodborne salmonellosis outbreak due to powdered infant formula contaminated with lactose-fermenting *Salmonella virchow.* Eur J Epidemiol 1996; 12: 377–81.

29) . Weber, D.J., Saviteer, S.M., Rutala, W.A., and Thomann, C.A. (1988) In vitro susceptibility of *Bacillus* spp. to selected antimicrobial agents. Antimicrob.agents Chemother., 32 : 642-645.

30) . de la Rosa, M.C., Mosso, M.A., Garcia, M.L., and Plaza, C. (1993) Resistance to the antimicrobial agents of bacteria isolated from non-sterile pharmaceuticals. J.Appl.Microbiol., 74: 570-577.

31) . Cormican, M.G. and Jones, R.N. (1996) Emerging resistance to antimicrobial agents in Gram-positive bacteria ; enterococci; staphylococci and non-pneumococcal streptococci . Drugs, 51 (suppl.1): 6-12 .

32) . Jacoby, G.A. (1996) Antimicrobial-resistant pathogens in the 1990s . Annu.Rev Med., 47 : 169-179.

33) . Jones, R.N. (2001) Resistance patterns among nosocomial pathogens, trends over the past few years.Chest,119 : 397S- 404S .

34) . Davies, A.L. and Stone, J.W. (1986) Current problems of chemotherapy of infections with coagulase – negative staphylococci. Europ. J. Clin. Microbiol., 5 : 277- 281.

35) . Courvalin., P. (1994) Transfer of antibiotic resistance between Gram-positive and Gram-negative bacteria. Antimicrob.Agents Chemother., 38: 1447-1451.

36) . Mazodier, P. and Davies, J. (1991) Gene transfer between distantly related bacteria. Ann.Rev.Genet., 25: 147-171.

37) . Schaberg, D.R. and Zervos, M.J. (1986) Intergeneric and interspecies gene exchange in Gram-positive cocci. Antimicrob. Agents Chemother., 30: 817-822.

38) . Al-Charrakh, A.H. (2000) Antibiotic resistance of *Klebsiella* , *Enterobacter*, and *Serratia* strains isolated from clinical specimens in Najaf. Iraq. J. Babylon Univ., 5 (4):478-482.

39) . Jarlier, V., Fosse, T., and Philipon, A. (1996) Antibiotic susceptibility in aerobic Gram- negative bacilli isolated in intensive care units in 39 French teaching hospitals (ICU study) Intensive Care Med., 22:1057- 1065.

40) . Wiedemann, B. (1996) Epidemiology, control, and treatment of multiresistant Gram- negative rods. Drugs,52 (suppl. 2) : 95-102 .

41) . Chanal, C., Sirot, D., Romaszko, J.P., Bret, L.,and Sirot, J. (1996) Survey of prevalence of extended spectrum ß-lactamases among Enterobacteriaceae. J.Antimicrob.Chemother.,38 : 127-132.

42) . Piddock, L.J.V., Walters, R.N., Jin, Y.F., Turner, H.L.,Gascoyne-Binsi, D.M., and Hawkey, P. M. (1997) Prevalence and mechanism of resistance to third-generation cephalosporins in clinically relevant isolates of Enterobacteriaceae from 43 hospitals in the UK, 1990-1991. J.Antimicrob. Chemother., 39:177-187.

43) . Bush, K., Jacoby, G.A., and Mederios, A.A. (1995) A functional classification scheme for ß-lactamses and its correlation with molecular structure . Antimicrob. Agents Chemother., 39 : 1211-1233.

44) .Thomson, K.S., Sanders, C.C., and Washington, J.A. (1991) High-level resistance to cefotaxime and ceftazidime in *Klebsiella pneumoniae* isolates from Cleveland, Ohio. Antimicrob.Agents Chemother., 35 : 1001-1005 .

Table 1 Types and numbers of bacterial species recovered from PIF samples.

Bacterial species	No. of isolates (%)
G+ve bacteria species:	
Staphylococcus aureus	6 (17.1)
Staphylococcus epidermidis	9 (25.7)
Bacillus subtilis	9 (25.7)
Bacillus spp.	3 (8.5)
G-ve bacteria species:	
Klebsiella pneumoniae,	3 (8.5)
Klebsiella spp.	2 (5.7)
Enterobacter cloacae	3 (8.5)
Citrobacter freundii.	2 (5.7)
Enterobacter sakazaki,	1 (2.8)
Total	38 (100)

Table 2 Percentages of resistance to antimicrobial agents of G+ve bacterial species recovered from PIF samples.

Bacterial isolate (No.)	**% of resistance to indicated drug**															
	P	AMP	Amx	CN	CTX	CZX	AMC	PB	B	GN	SM	E	C	R	Cp	SXT
Staphylococcus aureus (6)	1000	1000	1000	66	83	66	83	50	33	83	50	83	66	16	33	66
Staph .epidermidis (9)	1000	1000	1000	66	66	66	22	55	44	77	44	55	44	0	16	55
Bacillus subtilis (3)	33	33	66	0	66	33	0	33	33	66	33	66	33	0	0	66
Bacuillus spp. (6)	50	83	66	33	50	66	33	33	16	66	33	83	66	0	16	66

Abbreviations: Penicillin (P), Ampicillin (Amp), Amoxicillin (Amx), Cephalothin (CN), Cefotaxime (Ctx), Ceftizoxime (Czx), Amoxicillin/ Clavulanic acid (AMC), Gentamycin (Gn), Streptomycin (S), Erythromycin (E), Chloramphenicol (C),Tetracycline (Te), Lincomycin (L), Rifampicin (R), Polymyxin B (PB), Bacitracin (B), Ciprofluxacin (Cp) and Trimethoprim–Sufamethoxazole (SXT).

Table-3: Resistance to antimicrobial agents of Gram-ve bacterial species isolated from PIF samples.

Bacterial isolate	Resistance (+) to indicated drug															
	P	AMP	Amx	CN	CTX	CZX	AMC	PB	B	GN	SM	E	C	R	Cp	SXT
Klebsiella pneumoniae, 1	+	+	+	+	+	+	+	-	+	+	-	+	-	-	-	+
K. pneumoniae,2	+	+	+	-	+	-	-	+	-	+	-	+	-	-	+	+
K.pneumoniae, 3	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	-
Klebsiella spp. 1	+	+	+	+	-	+	-	+	+	+	+	+	+	-	-	-
Klebsiella spp. 2	+	+	+	-	-	+	+	-	-	-	-	-	+	-	-	+
Enterobacter cloacae,1	+	+	-	+	+	-	-	+	-	+	+	+	+	-	+	+
En.cloacae,2	+	+	-	-	+	+	+	-	+	+	-	+	-	+	-	+
En. cloacae, 3	+	+	-	-	+	+	+	-	+	+	+	-	+	-	-	-
Citrobacter freundii1	+	+	-	+	+	-	+	-	+	-	-	+	-	-	+	+
Citrobacter freundii.2	+	+	+	-	-	+	-	+	+	-	-	+	-	-	+	-
Enterobacter sakazaki, 1	+	+	+	+	+	-	+	+	-	+	+	-	+	+	-	+
	+	+	-	-	-	+	+	+	-	+	-	+	-	-	-	-
	+	-	+	+	+	-	+	-	+	-	-	+	+	-	+	+
	+	-	-	-	-	-	-	-	+	+	-	+	-	-	-	+

Abbreviations: Penicillin (P), Ampicillin (Amp), Amoxicillin (Amx), Cephalothin (CN), Cefotaxime (Ctx), Ceftizoxime (Czx), Amoxicillin/ Clavulanic acid (AMC), Gentamycin (Gn), Streptomycin (S), Erythromycin (E), Chloramphenicol (C),Tetracycline (Te), Lincomycin (L), Rifampicin (R), Polymyxin B (PB), Bacitracin (B), Ciprofluxacin (Cp) and Trimethoprim–Sufamethoxazole (SXT).