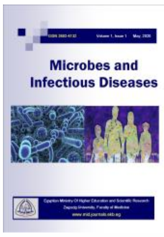
Microbes and Infectious Diseases 2022; Article-In-Press, **DOI:** 10.21608/mid.2022.137933.1313



Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Antibiotic resistance of *Bacillus* species isolated from hawked 'suya' meat sold in Kaduna metropolis, North-Western Nigeria

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ARTICLE INFO

Article history:
Received 11 May 2022
Received in revised form 2 June 2022
Accepted 6 June 2022

Keywords:
Food
Pathogens
Occurrence
Resistance
Antibiotics

ABSTRACT

Background: Food poisoning and antibiotic resistance among bacterial community has become a serious global challenge in the treatment of infections cause by pathogens. This research work was carried out to determine the occurrence and antibiotic resistance of *Bacillus* species in 200 hawked 'suya' meat sold in Kaduna metropolis. **Methods:** *Bacillus* species were isolated using a selective medium and biochemical tests. The isolates were further identified using Microgen Bacillus ID identification kit and the antibiotic resistance pattern was determined using single disc diffusion technique on Mueller-Hinton agar. **Results:** The total percentage occurrence of *Bacillus* species in the metropolis was 34.0% (68/200) with *Bacillus cereus* having the highest occurrence of 39.7% (27/68) and *Bacillus lentus* having the lowest occurrence of 1.5% (1/68). The multiple antibiotic resistance (MAR) index of the *Bacillus* species indicates that 72.50% (29/40), 50.0% (29/58), 47.3% (29/58) of *Bacillus cereus*, *Bacillus subtilis* and *Bacillus megaterium* respectively had MAR index above 0.20 significant level of MAR of resistant pathogens. **Conclusion:** The presence of *Bacillus* species in the suya gives a warning signal for possible occurrence of foodborne infections and capable of producing outbreak of food poisoning. The multiple antibiotic resistance of *Bacillus* species calls for concern.

Introduction

In the globe today, different countries traditionally processed meat into various meat products [1]. In Nigeria, one of the meat products mostly consumed is suya. "Suya" is a traditional meat product gotten from boneless meat usually suya is made from beef, ram and goat meat which is hang on stick and spiced or marinated in groundnut cake, salt, vegetable oil and other flavours followed by roasting around a glowing charcoal fire [2]. Suya originated from the northern parts of Nigeria, it has permeated Nigerian society being affordable for all and available everywhere [3]. It has become very popular as a street delicacy in several countries, in parts of Nigeria and generally in West Africa [3].

However, the preparation process is usually carried out under unhygienic conditions and the risk of contamination is very high [4,2]. Most suya are often displayed in Nigeria markets under poor hygienic conditions and hence contaminated by various microorganisms [1]. Most of the suya are vended by the streets on uncovered trays where dusts containing microbes are raised by vehicles or humans' activities and contaminate the meat product [3].

DOI: 10.21608/MID.2022.137933.1313

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Consumption of food that contains *Bacillus* species especially *Bacillus cereus* may result in food poisoning through consumption of food containing pre-formed toxin or toxins produced by these bacteria in the human gut [5,6]. The *B. cereus* groups which are known pathogens or opportunistic pathogens to humans are closely related to *Bacillus anthracis*, *Bacillus thuringiensis* and *Bacillus mycoides* [6].

Antimicrobial resistance is a growing problem around the world and is associated with increasing mortality and medical costs. This study therefore, sought to assess the occurrence and determination of antibiotic pattern of *Bacillus* species in suya which can be of public health importance [7, 6].

Materials and Methods

Food samples

A total of 200 suya samples were collected from hawkers and 50 samples were apportioned evenly to the four different markets and were purchased randomly. All the samples were collected during dry season and transported in a sampling box containing ice pack to National Research Institute for Chemical Technology, Zaria for microbial analysis.

Sample preparation, isolation and phenotypic characterization of *Bacillus* species

Twenty-five gram (25g) of homogenised food sample was inoculated in 225 ml of buffered bacteriological peptone water and incubated for 24 h at ambient temperature [8]. A loop full of the culture from the enrichment broth was sub-cultured on mannitol egg yolk polymyxin agar plates and incubated at 37°C for 24 h. Typical colonies of *Bacillus* species were identified by biochemical characterization that includes Gram staining, motility, haemolysis, Voges-Prokauer, oxidase, methyl red, nitrate reduction, citrate utilization, indole, coagulase, and urease tests as suggested by **Adekanmi et al.** [1]. All the isolates were further confirmed using a Microgen® Bacillus ID identification kit (Microgen Bioproducts, U.K.).

Antibiotic susceptibility test

The antibiotic susceptibility pattern was determined using Kirby-Bauer-NCCLS modified single disc diffusion technique on Mueller-Hinton agar [6]. Single antibiotic disc such as ampicillin (10μg), vancomycin (30μg), tetracycline (30μg), clindamycin (2μg), erythromycin (15μg), ciprofloxacin (5μg), penicillin G (10μg), gentamycin (10μg), amoxacillin/clavulate (30μg), chloramphenicol (30μg), cefoxitin (30μg) and oxacillin (1μg) Oxoid, England was used to determine the antibiotic susceptibility of the isolates. The zones of inhibitions were measured and the results were interpreted using the guideline from **CLSI** [9].

Determination of multiple antibiotics resistance (MAR) index

Multiple antibiotics resistance determined using the formula MAR = X/Y where X is the number of antibiotics to whish the test isolates displayed resistance to and Y is the total number of antibiotics to which the test organism has been evaluated for sensitivity [10].

Results

The phenotypic characterization of the *Bacillus* species using microgen identification system as in **Table 1** showed that the percentage identification of *Bacillus cereus* group ranged from 97.88 to 99.99%, more of the isolates were identified at 99.88% and 99.99%. *Bacillus megaterium* were mostly identified at 99.99%. *Bacillus subtilis* were identified at 99.93 and 99.68%.

The total occurrence of *Bacillus* species in the four sampling locations was 34.0% (68/200). The total occurrence of *Bacillus subtilis* from the four locations was 45.6% (31/68) and the total percentage occurrence of *Bacillus cereus* was 39.7% (27/86). *Bacillus megaterium* and *Bacillus lentus* had total occurrence of 13.2% (9/68) and 1.5% (1/68) respectively as presented in **figure (1)**. The total number of *Bacillus* species isolated from each of the selected market showed that 48.0% (24/50) were from Kawo Market, 18% (9/50) from Kakuri Market, 24.0% (12/50) from Ungwan Boro Market and 46.0% (23/50) from Tudun wada Market.

The antibiotic susceptibility of *Bacillus* species showed that all the isolates of *Bacillus* species were 100% susceptible to vancomycin and isolates of *Bacillus cereus* were 100% susceptible to clindamycin. The *Bacillus* species isolated had high percentage resistant to ampicillin, oxacillin, cefoxitin, penicillin and erythromycin **(Table 2)**. One of the isolates (i.e. *Bacillus lentus*) was susceptible to all the antibiotics selected for the research.

The antibiotic resistance pattern of the resistant isolates as presented in **table (3)** showed that there was much disparity of wide range in resistance of *Bacillus* species to the antibiotics selected for the research. A total of twenty isolates of *Bacillus* species were resistant to four classes of antibiotics, and a total of sixty-four isolates were resistant to three classes of antibiotics. The total MAR index of the *Bacillus* species indicates that 72.50% (29/40), 50.0% (29/58), 47.3% (29/58) of *Bacillus cereus*, *Bacillus subtilis* and *Bacillus megaterium* respectively had MAR index above 0.20 significant level of MAR of resistance pathogens **(Table 4)**.

Table 1. Phenotypic characterization of *Bacillus* species using Microgen identification kit

S/NO	ARA	CEL	INO	MAN	MNS	RAF	RAH	SAL	SOR	SUC	TRE	XYL	ADO	GAL	MDM	MAG	INU	MLZ	IND	ONPG	ARG	CIT	VP	NIT	Octal Code	Prob-ability (%)	inference Organism (No.)
1	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-	+	-	-	+	-	+	+	**00221113**	**99.88**	***B. cereus* Group(8)**
2	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	+	-	+	+	**00220033**	**96.16**	***B. cereus* Group(4)**
3	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	**00260012**	**99.07**	***B. cereus* Group(3)**
4	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-	**00220110**	**99.96**	***B. cereus* Group(4)**
5	-	+	-	-	-	+	-	-	+	-	+	+	-	+	-	-	+	-	-	-	-	-	-	-	**21332200**	**98.59**	***B. megaterium*(1)**
6	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	+	-	-	+	**00220031**	**97.88**	***B. cereus* Group(2)**
7	-	-	+	-	-	+	-	-	+	-	-	-	-	+	-	-	+	+	-	+	+	-	-	+	**11102331**	**99.99**	***B. megaterium*(5)**
8	+	-	-	-	-	-	-	-	+	-	-	+	-	+	-	-	+	+	-	+	+	-	-	+	**40112331**	**99.92**	***B. megaterium*(3)**
9	-	+	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-	**30220110**	**99.99**	***B. cereus* Group(6)**
10	-	+	+	-	+	+	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	+	-	**77400444**	**99.99**	***B. lentus*(2)**
11	+	+	+	+	+	-	-	+	+	+	+	+	-	-	-	+	-	-	-	-	-	+	+	+	**76370407**	**99.93**	***B. subtilis*(14)**
12	+	+	-	+	+	-	-	+	+	+	+	+	-	-	-	+	-	-	-	-	-	+	+	+	**66370407**	**99.68**	***B. subtilis*(17)**

ARA = Arabinose, CEL = Cellobiose, INO = Inositol, MAN = Mannitol, MNS = Mannose, RAf = Raffinose, RHA = Rhamnose, SAL = Salicin, SOR = Sorbitol, SUC = Sucrose, TRE = Trehalose, XYL = Xylose, ADO = Adonitol, GAL = Galactose, MDM = Methyl-D-mannoside, MAG = Methyl-D-Glucose, INU = Inulin, MLZ = Melezitose, IND = Indole, ONPG = Ortho-Nitrophenol-β-galactoside, ARG = Argenine, CIT = Citrate utilization, VP = Voges Proskauer, NIT = Nitrate.

Table 2. Antibiotic susceptibility of *Bacillus* species isolated from the hawked suya (roasted) meat

S/N	Antibiotics	*Bacillus cereus* (n = 27)			*Bacillus megaterium* (n=9)			*Bacillus subtilis* (n=31)		
		R(%)	I(%)	S(%)	R(%)	I(%)	S(%)	R(%)	I(%)	S(%)
1	Vancomycin (30 µg)	0(0)	0(0)	27(100)	0(0)	0(0)	9(100)	0(0)	0(0)	31(100)
2	Clindamycin (2 µg)	0(0)	0(0)	27(100)	0(0)	1(11)	8(89)	5(16)	7(23)	19(61)
3	Erythromycin (15 µg)	18(67)	5(19)	4(15)	5(56)	2(22)	2(22)	25(81)	2(6)	4(13)
4	Amoxicillin/Clavulate(30 µg)	7(26)	-	20(74)	3(33)	-	6(67)	15(48)	-	16(52)
5	Cefoxitin (30 µg)	16(59)	-	11(41)	7(78)	-	2(22)	21(68)	-	10(32)
6	Chloramphenicol (30 µg)	15(56)	6(22)	6(22)	5(56)	1(11)	3(33)	22(71)	3(10)	6(19)
7	Ampicillin (10 µg)	23(85)	-	4(15)	8(89)	-	1(11)	26(84)	-	5(16)
8	Tetracycline (30 µg)	24(89)	1(4)	2(7)	5(56)	2(22)	2(22)	22(71)	4(13)	5(16)
9	Gentamicin (10 µg)	19(70)	3(11)	5(19)	4(44)	2(22)	3(33)	21(68)	4(13)	6(19)
10	Oxacillin (1 µg)	21(78)	-	6(22)	6(67)	-	3(33)	24(77)	-	7(23)
11	Ciprofloxacin (5 µg)	15(56)	5(19)	7(26)	4(44)	3(33)	2(22)	16(52)	5(16)	10(32)
12	Penicillin (10 µg)	27(100)	0(0)	0(0)	8(89)	0(0)	1(11)	30(97)	1(3)	0(0)

Table 3. Phenotypic antibiotic resistance patterns of the *Bacillus* species.

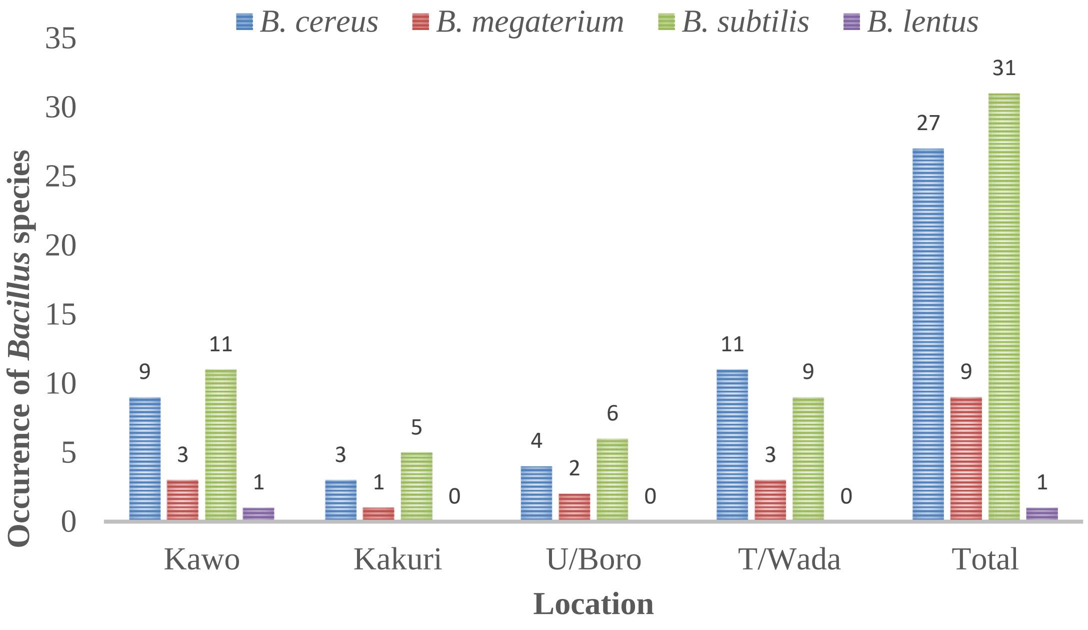
S/N	*Bacillus cereus*		*Bacillus megaterium*		*Bacillus subtilis*	
	Resistance pattern	Frequency	Resistance pattern	Frequency	Resistance pattern	Frequency
1	E	6	C	3	E	8
2	TE	4	OX	2	DA	5
3	P	7	AMP	3	TE	5
4	AMP	8	P	3	P	16
5	C, CIP	3	FOX	3	AMP	6
6	TE, OX	4	E, CN	2	FOX, C	5
7	FOX, CN	1	C, OX, AMP	1	CN, OX	8
8	E, TE, P	4	AMC, TE, CIP	3	AMC, E	4
9	OX, CN, CIP	2	OX, TE, C, CN	1	TE, AMP	6
10	AMP, FOX, C	3	AMP, E, P, FOX	3	CIP, P, E	5
11	P, CN, E, CIP	3	CN, OX, P, AMP	1	E, CIP, P	5
12	AMC, E, TE, P	4	FOX,CIP,P,TE, OX	1	TE, AMC, FOX	6
13	OX, FOX, CN, CIP	7			C, CIP, P, OX	1
14	CN, C, OX, TE, FOX	5			FOX, AMP, C, CN	7
15	OX, CN, AMP, C, E	1			CN, TE, AMC, OX	3
16	AMC, AMP,TE, P, C	3			CIP, OX, TE, C, AMC	2
					P, OX, FOX, E, CN, CIP	3

KEY: E = Erythromycin, TE = Tetracycline, P = Penicillin, C = Chloramphenicol, OX = Oxacillin, AMP = Ampicillin, FOX = Cefoxitin, CN = Gentamycin, AMC = Amoxicillin/Clavulate, CIP = Ciprofloxacin

Table 4. Multiple antibiotic resistant (MAR) indices of the isolates of *Bacillus* species.

Organism	No. of multidrug resistant isolates	Classes of antibiotics to which isolates were resistant to	MAR index	Total MAR index of isolates greater than 0.20 significant value (%)
Bacillus cereus	11	2	0.17	-
	20	3	0.25	72.5
	9	4	0.33	
Bacillus megaterium	29	2	0.17	-
	23	3	0.25	47.3
	3	4	0.33	
Bacillus subtilis	29	2	0.17	-
	21	3	0.25	50.0
	8	4	0.33	

Figure 1. Occurrence of *Bacillus* species in hawked suya from the selected market.



Key: U/Boro = Ungwan Boro, T/Wada = Tudun Wada

Discussion

The presence of *Bacillus* species in the suya samples may be due to contamination from long exposure to air during hawking as reported by **Adesoji et al.** [11] and this air might have polluted with different types of microorganisms. The presence of *Bacillus cereus* is of public health importance because it is frequently associated with cases of food poisonings as reported by **Agwa et al.** [12] and **Osman et al.** [6]. This could be as results of toxins produced by the organism. Food poisoning caused by Bacillus *cereus* occurs year-round diffusely over a geographic area [6].

Suya may serve as a bed for multiplication of *Bacillus* species because of poor roasting and can result to be a major threat to human populations or public health as reported by **Orogu and Oshilim** [7]. Suya can serve as potential bed for multiplication of *Bacillus* species because it is rich in nitrogenous compounds (amino acids, peptides, proteins), minerals and other growth factors as reported by **El-Hersh et al.** [13]. In addition, they have some fermentable carbohydrates, usually glycogen and keep favourable pH for growth of most microorganisms [14].

These constituents promote the growth and multiplication of various organisms in meat borne pathogens such as aerobic spore formers (e.g., *Bacillus* species) that may constitute public health hazards [15,16]. It is not surprising that *Bacillus* species were isolated from suya because these bacteria can survive heat during roasting; this is because *Bacillus* species can form spores to resist high temperature [8,1]. The spores can grow to vegetative form once there are favourable environmental conditions [15].

The presence of antibiotic-resistant *Bacillus* species in the food samples has an important health implication, since there is frequent and uncontrolled use of antibiotics in both veterinary and human medicine [17]. Antibiotic-resistance among pathogens usually interfere with effective treatment measures of diseases cause by the infectious agents [18].

Detection of multidrug resistant pathogenic bacteria in foods is therefore considered as a public health threat [19]. Excessive application of antibiotics in both human and veterinary medicine may lead to distribution of antibiotic-resistant pathogens in foods and environment. Multiple antibiotic resistances in *Bacillus* species might be attributed to antimicrobial selective pressure and gene transfer mechanisms between and among *Bacillus* species [20].

Multiple antibiotic resistance index value lower than 0.20 indicates that the *Bacillus* species might have originated from a lower risk source in which the antibiotics are seldom or never used [21]. Multiple antibiotic resistance index value higher than 0.20 indicates that the isolates of *Bacillus* species might have originated from the environment where there is indiscriminate use of the antibiotics. This calls for concern because it is an alert that the antibiotics might have been abused in environment where *Bacillus* species were isolated.

Conclusion

The overall occurrence of the *Bacillus* species in the food samples is 34.0% (68/200), *Bacillus subtilis* had highest occurrence of 45.6% (31/68) and *Bacillus lentus* had the least occurrence of 1.5% (1/68). Twenty (20) isolates of *Bacillus* species were resistant to four classes of antibiotics, and sixty-four (64) isolates were resistant to three classes of antibiotics. It is therefore, recommended that hawkers should ensure that their suya is well roasted and protected from contamination to avoid foodborne infections associated with *Bacillus* species.

Funding: None

Competing interests: No competing interest declared

References

1- **Adekanmi AA, Dapo OA, Adekanmi AS, Adkunle MT.** Heat Resistance Bacillus species isolated from Tsire-Suya. International Journal of Academic and Applied Research 2020; 4(5):31-46.

2- **Orpin JB, Mzungu I, Osuji CG.** Isolation and Identification of Bacteria Associated with Suya (Roasted Meat Product) Sold in Dutsinma Local Government Area, Kastina State. Journal of Advances in Biology & Biotechnology 2018; 20(2):1-8.

3- **Eke SO, Irabor JI, Okoye M, Aitufe OF, Ekoh SN.** The microbial status of commercial ‘suya’ meat products in Ekpoma, Edo, Nigeria. International Journal of Community Research 2013; 2(1):18-21.

4- **Falegan CR, Akoja SO, Oyarekua MA.** Microbiological assessment of suya (sliced roasted beef) in Ado-Ekiti Metropolis, Ekiti State, Nigeria. MOJ Biology and Medicine 2017; 2(3):266-269.

5- **Guven K, Mutlu MB, Avci O.** Incidence and Characterization of *Bacillus cereus* in Meat and Meat Products Consumed in Turkey, Journal of Food Safety 2006; 26:30-40.

6- **Osman KM, Kappell AD, Orabi1 A, Al-Maary KS, Mubarak AS, Dawoud TM, et al.** Poultry and beef meat as potential seedbeds for antimicrobial resistant enterotoxigenic *Bacillus* species: a materializing epidemiological and potential severe health hazard. Scientific Reports 2018; 8:11600.

7- **Orogu JO, Oshilim AO.** Comparative study of bacteriological analysis in hawked suya meat and suya meat on a Barbeque stand. Journal of Microbiology Research 2017; 3(1):005-008.

8- **Amaeze N, Aboh MI, Amiohu FE, Olatunji T, Oladosu P.** Microbial Profile, Antibiotic Sensitivity and Heat Resistance of Bacterial Isolates from Commercial Roasted Beef (Suya) in Abuja, Nigeria. Journal of Phytomedicine and Therapeutic 2016; 15(2):22-30.

9- **Clinical and Laboratory Standards Institute, CLSI.** Performance standards for antimicrobial susceptibility testing, 26th Edition, M100S. Wayne, PA, 2021; 36(1):52-115.

10- **Tula MY, Okoro AV, Okojie RO, Iyoha O.** Antimicrobial susceptibility pattern and plasmid-mediated antibacterial resistance in *Staphylococcus aureus* and coagulase-negative Staphylococci (CoNS). American Journal of Research Communication 2016; **1**(9):149-166.

11- **Adesoji AT, Onuh JP, Musa AO, Akinrosoye PF.** Bacteriological qualities and antibiogram studies of bacteria from "suya" and smoked fish (*Clarias gariepinus*) in Dutsin-Ma, Katsina State, Nigeria. Pan African Medical Journal 2019; 33:219.

12-**Agwa OK, Uzoigwe CI, Wokoma EC.** Incidence and Antibiotic Sensitivity of *Bacillus cereus* Isolated from Ready-to-eat Foods Sold in Some Markets in PortHarcourt, Rivers State, Nigeria. Asian Journal of Microbiology Biotechnology Environmental Science 2012; 14(1):13-18.

13-**El-Hersh MS**, **Saber WIA, El- Fadaly HA.** Amino Acids Associated with Optimized Alkaline Protease Production by *Bacillus subtilis* ATCC 11774 Using Statistical Approach. Journal of Biotechnology 2014; 13(6):252-262.

14-**Bermudez R, Lorenzo JM, Fonseca S, Franco I, Carballo J.** Strains of *Staphylococcus* and *Bacillus* isolated from traditional sausages as producers of biogenic amines. *Frontiers in Microbiology*, 2012; 3(151):1-6.

15-**Hemmat MI, Amani MS, Dalia AS, Ghada AA.** Demonstration of aerobic spore formers in some meat products. Benha veterinary medical journal 2014; 26(2):219-226.

16-**Eruteya OC, Nmehielle E-PJ.** Assessment of the Safety of ‘Suya’ Condiment Vended in Three Communities in Rivers State, Nigeria. *International Journal of Pathogen Research*, 2021; 6(1):1-6.

17-**Beric T, Biocanin M, Stankovic S, Dimkić I, Janakiev T, Fira Đ, et al.** Identification and antibiotic resistance of *Bacillus* spp. isolates from natural samples. Arch Biological Science 2018; 70(3):581-588.

18-**von Wintersdorff CJH, Penders J, van Niekerk JM, Mills ND, Majumder S, Van Alphen LBet al.** Dissemination of Antimicrobial Resistance in Microbial Ecosystems through Horizontal Gene Transfer. Frontiers in Microbiology 2016; 7:173.

19-**Kemal M.** Antibiotics Misuse and factors leading to its’ abuse in Kurdistan Region. Journal of Health, Medicine and Nursing 2016; 24:20-27.

20-**Peterson E, Kaur P.** Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. Frontiers in Microbiology 2018; 9:2928.

21-**Osundiya OO, Oladele RO, Oduyebo OO.** Multiple Antibiotic Resistance (MAR) Indices of Pseudomonas and Klebsiella species Isolates in Lagos University Teaching Hospital. African Journal of Clinical and Experimental Microbiology 2013; 14(3):164-168.