High prevalence of Antibiotic Resistance Bacteria isolated from Municipal Solid Waste Dumpsite, Bahir Dar, Ethiopia

Baye Sitotaw (✉ mershabaye@gmail.com)
  Bahir Dar University

Fikremariam Ayalew
  Bahir Dar University

Abayeneh Girma
  Mekdela Amba University

Kindu Geta
  Debre Tabor University

Mulugeta Kibret
  Bahir Dar University

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Abstract

Background The emergence of antibiotic resistance (ABR) among environmental microbes has been challenging global health. ABR can be transferred to human-associated bacteria aggravating the spread of antimicrobial resistance. Due to poor solid waste disposal practices, municipal solid waste dumpsite (MSWDS) can be a reservoir for antimicrobial resistant microorganisms, such as bacteria. This study aimed to assess the prevalence of antibiotic resistance (ABR) bacteria at Bahir Dar city MSWDS. Methods Soil samples were collected from three randomly selected sites within the dumpsite from November 2020 to May 2021. Bacteria were isolated, identified and tested for ABR using standard procedures. Results In this study, 71 distinct colonies were isolated and identified to ten bacterial genera based on cultural characteristics and biochemical tests. The pooled antibiotic résistance rate was 85.9%. Resistance to the tested antibiotics ranged between 0% for erythromycin and 100% for amoxicillin among the bacterial isolates. High proportions of the isolates were found to be resistant to amoxicillin (100%), vancomycin (87%) and nalidixic acid (73.3%). Substantial proportions of the isolates were also resistant to Streptomycin (54.5%), sulfonamide (50%) and tetracycline (48.5%). On the other hand, high sensitivity rates to erythromycin (90.91%), ciprofloxacin (83.10%), chloramphenicol (77.46%) and gentamicin (63.49%) were recorded. All isolates related to Staphylococcus spp., and most isolates related to Pseudomonas, Escherichia, Klebsiella, Salmonella, Enterococcus, Citrobacter, Shigella and Proteus species were resistant at least to one antibiotic drug. The overall multidrug resistance (MDR) rate was 49.3%, and a high rate of MDR was demonstrated among isolates related to Escherichia spp. (75%), Staphylococcus spp. (68.8%), Pseudomonas spp. (62.5%) and Klebsiella spp. (60%), where each isolate resisted at least 5 antibiotic drugs. Conclusion From the present study, it can be concluded that a high incidence of ABR exists in Bahir Dar city MSWDS. The occurrence of the high level of ABR to commonly used antibiotics in this study demands a proper waste management system, as well as surveillance programs to monitor for antimicrobial resistance determinants in municipal solid wastes. Moreover, detailed studies on the isolates and ABR genes will give a better insight into the prevalence of ABR in the waste dumpsite.

Introduction

Antimicrobial resistance has been a top threat to public health, and a priority concern to global health [1, 2]. Bacterial pathogens are among the leading pathogenic microorganisms and have been posing serious public health problems by developing antibiotic resistance (ABR). The rate of increase in resistant bacteria to antibiotics necessitates the search for novel antibiotics. Continued efforts in the understanding of the ecology and resistance patterns of bacteria are important for tackling the associated disease burden at the national and global levels. Most studies regarding the prevalence of ABR have long been focused on samples from health institutions (clinical environment) [3]. Later, studies on the natural resistome or from non-clinical environments are also considered important [4, 5, 6]. The soil environments, particularly waste dumpsite soils are scarcely investigated for ABR. However, growing interests are shown in the understanding of natural antibiotic resistome [1, 7]. Environmental resistome

can be a reservoir of resistance genes from which pathogenic bacteria could gain antibiotic resistance genes [7].

Municipal solid waste dumpsites (MSWDS), which refers to areas where solid wastes from several sources are deposited, are a potential source of drug resistance genes [8, 9, 10, 11, 12]. Worldwide, the indiscriminate and continuous dumping of solid wastes introduces several hazardous items such as heavy metals, expired pharmaceutical wastes, residual antimicrobial agents and pathogenic microorganisms that are thrown into a common dumping site irresponsibly or without knowing their risks when dumped untreated [13, 14, 15]. Particularly, expired pharmaceuticals and residual antimicrobial agents that are discharged randomly into the dumpsite pose a selection pressure in favor of resistant bacteria by killing or inhibiting the growth of susceptible ones [16], which often results in mutations in the bacterial population. This leads to the mass increase in resistant bacteria as the competing normal flora will be wiped out by the dumped pollutants [17]. The resistant strains of bacteria can also be accelerated and spread by the transfer of resistant genes among species and genera through horizontal gene transfer by various mechanisms, such as mobile genetic elements. All of these processes can lead to the development and dissemination of new antimicrobial resistance variants with novel resistance mechanisms against various classes of commonly used antimicrobial agents.

Currently, several authors have reported the prevalence of bacteria of public health importance from MSWDS [18, 19, 20, 21, 22]. Several ABR enteric bacteria were identified from dumpsite by Mwaikono et al. [21]. A recent study by Hrenovic et al. [22] further demonstrated that, of all types of environment the waste dumpsite soil probably harbors the largest and most diverse resistome including both bacteria with intrinsic and acquired ABR.

Despite the huge volume of solid waste generation in municipalities of underdeveloped countries like Ethiopia, limited research is available regarding antimicrobial-resistant bacteria from dumpsites. The resistance rates and resistance mechanisms are influenced by geographic variations [23]. The interplay of several determinant factors within a locality greatly varies. The local abiotic and biotic factors and the types of waste generated vary from place to place. Microbial proliferation thus depends on several factors, such as local environmental conditions and types and the amount of available nutrients. It is thus logical that risks to public health from wastes in one municipal dumpsite may not be the same elsewhere [24]. Therefore, ABR profiles of pathogenic bacteria may vary from location to location, and can also change rapidly with time; as such, they need to be monitored and managed closely because of their public health implications. This study thus aimed to investigate the prevalence of antibiotic resistant bacteria in Bahir Dar city MSWDS.

Methods

Study area description

This study was conducted in Bahir Dar City (northwest Ethiopia), which is some 565 km far from Addis Ababa (the capital of Ethiopia). The study site, the main solid waste disposal site in Bahir Dar city is

located between 11° 32' 23" to 11° 32' 37" N latitude and 37° 23' 12" to 37° 23' 24" E longitude. It is situated at about 7 km outskirts of the city at an elevation of 1,790 meters above sea levels and covers an area of about 22 hectares. The area has a warm temperature with a mean annual temperature of 13.5 to 27.7 °C and a mean annual rainfall of about 1500 mm of which 54% of the fall is in July and August when the rainfall can reach 250-300 mm per month.

Bahir Dar city generates more than 98.8 tons of waste daily [25]. The waste is a mixture of residential (54 %), commercial (24.2 %), institutional (17 %), and street sweeping (3.56 %) [25]. There is an unrestricted dumping process and due to that plenty of scattered pharmaceutical drugs can be observed at the dumping.

Soil sample collection

Three sampling sites were randomly selected within Bahir Dar city MSWDS. After removing all surface debris, the site was dug into 4-6 cm and soil samples (50 g from each site) were collected with a sterile spatula and placed into sterilized zip-lock polythene bags. The collected soil samples were transported to Bahir Dar University, Microbiology Laboratory in an icebox, and the culturing was begun immediately. The samples were collected bi-monthly from November 2020 to May 2021.

Isolation and identification of bacteria

A serial dilution technique was used to isolate the bacteria. Five gram of soil sample from each site was pooled to obtain one homogeneous sample. From the mix, 1g of homogenized soil sample was mixed with 9 ml of sterile normal saline solution (0.85 % NaCl) and vortexed for 1 minute to make a homogenous suspension. From the suspension, tenfold serial dilution was made and from the 10^{-5} to 10^{-9} dilutions, 0.1 ml was spread on nutrient agar (HiMedia, India) using a bent glass spreader. For each dilution, triplicate plates were used and incubated at 28 °C for 24-48 hours in an inverted position. Morphologically distinct bacterial colonies were collected and purified on the same medium by the streak plate method. The pure isolates were maintained in nutrient broth (HiMedia, India) at 4°C for identification and antibiotic susceptibility test. The isolates were first categorized based on their colony characteristics and Gram staining, and identified by several biochemical tests as described in Bergey's Manual of Determinative Bacteriology [26, 27].

Antimicrobial susceptibility test on bacterial isolates

After identifying the bacterial isolates, the standard Kirby-Bauer`s disc diffusion method was performed to determine their antimicrobial resistance profiles following standard procedures [28, 29]. Bacterial inoculum was prepared by suspending 4-5 morphologically identical colonies from each isolate in 5 ml nutrient broth (HiMedia, India) and incubated for 4 hours at 37°C. The bacterial suspension was compared with 0.5 McFarland turbidity standards. After adjusting the turbidity, the surface of the prepared Mueller Hinton Agar (MHA) medium (Accumix, India) was evenly inoculated three times using a

sterile cotton swab while rotating the plate with the culture. The plates were left at room temperature for 15-20 minutes to let dry.

The standard commercially available antibiotic discs used in this study were gentamicin (GN, 10 µg), streptomycin (ST, 30 µg), tetracycline (TE, 30 µg), ciprofloxacin (CIP, 5 µg), nalidixic acid (NA, 30 µg), sulfonamide (SA, 250 µg), chloramphenicol (C, 30 µg), erythromycin (E, 15 µg), vancomycin (V, 30 µg) and amoxicillin (AMX, 25 µg) (Becton, Dickinson, and Company, Sparks, Maryland, USA). The discs were aseptically laid on the surface of the inoculated agar plates with proper spacing using sterile forceps and incubated at 37°C for 18-24 hours. The diameter of the inhibition zone around the discs was measured to the nearest millimeter and interpreted as sensitive (S), intermediary resistant (I), or resistant (R) according to the defined breakpoints in Clinical and Laboratory Standards Institute [30].

There are no guideline breakpoints for *Bacillus* spp. in Clinical and Laboratory Standards Institute guidelines and the breakpoints of *Staphylococcus* spp. were used as criteria for interpretation. The breakpoints of enterobacteriales are recommended and used for other members in this family such as *Klebsiella, Escherichia, Citrobacter, Salmonella*, *Shigella* and *Proteus* species [30]. Similarly, the breakpoints of non-enterobacteriales, non-fastidious, glucose non-fermenter, Gram-negative and bacilli are recommended and used for *Pseudomonas spp.* except for *Pseudomonas aeruginosa.* For routine quality control of antimicrobial susceptibility tests, *Staphylococcus aureus* (ATCC® 25923) and *Escherichia coli* (ATCC® 25922) strains were used.

Data analysis and interpretation

Descriptive statistics, such as means and standard deviation are used to present the data. The antimicrobial susceptibility profiles of the bacterial isolates are reported as susceptible or sensitive (S), intermediary resistant (I), or resistant (R) according to the annually published microbiological breakpoints by [30]. The prevalence of multidrug resistance (MDR) isolates is also determined. MDR is when antimicrobial resistance that is shown by a species of microorganism to at least one antimicrobial drug in three or more antimicrobial categories [31]. The results are then presented in tables.

Limitation of the study

We understand that identification of the isolates to species level would be very important. Moreover, soil samples from the nearby environment where the soil is not affected by the dumped wastes should have been included to verify whether the source of the isolated bacteria is the municipal solid waste or not.

Results

Isolates and identification of bacteria

In this study, 71 distinct bacterial colonies were isolated and identified based on colony morphology and a series of other biochemical tests. Almost half of the isolates were Gram-negative, and they were further identified as one of the 10 genera (Additional file 1). Isolates related to *Staphylococcus* (23%) was the

most dominant followed by *Escherichia* (17%), *Bacillus* (14%), *Pseudomonas* (11%), *Enterococcus* (11%), *Salmonella* (9%), *Klebsiella* (7%), and *Citrobacter, Proteus and Shigella* (about 3% each) in that order. (Figure1).

Figurer 1.

Antimicrobial susceptibility profiles of the isolates

As shown in Table 1 and Additional file 2, most of the isolates were resistant to Amoxicillin (100%), Vancomycin (87%) and Nalidixic acid (73.3%). Moreover, almost half of the isolates were resistant to sulfonamide, tetracycline and Streptomycin. On the other hand, large proportions of the isolates were sensitive to erythromycin (90.9%), ciprofloxacin (83.1%), chloramphenicol (77.5%) and gentamicin (63.5%).

Table 1:

In this study, the overall antibiotic résistance rate was 85.9%. Isolates related to *Pseudomonas, Escherichia, Klebsiella, Salmonella, Enterococcus, Citrobacter, Shigella* and *Proteus* species were 100% resistance at least to one antibiotic drug. Similarly, 87.5% of the isolates related to *Staphylococcus* were resistant to at least one antibiotic drug. However, most of the isolates related to *Bacillus* spp. were sensitive to most of the tested drugs. Further, the overall multidrug resistance (MDR) rate was 49.3%, and a high rate of MDR was demonstrated among isolates related to *Escherichia* spp. (75%), *Staphylococcus* spp. (68.8%), *Pseudomonas* spp. (62.5%) and *Klebsiella* spp. (60%), and each isolate resisted at least 5 antibiotic drugs (Table 2, Additional file 2).

Table 2

Isolates related to *Staphylococcus* spp. demonstrated high rates of resistance to AMX, VA and NA; *Pseudomonas* spp. to AMX, NA, SA and TE; *Escherichia* spp. to AMX, NA and SA and *Klebsiella* spp. to SA, NA, TE and S (Additional file 2).

Discussion

Antibiotic-resistance (ABR) has been among the top concerns of global health care [2, 32]. Particularly, this problem can be big in low-income settings as the result of poor waste management systems, inadequate hygienic and sanitation practices [14, 33]. An in-depth study by Mwaikono et al.[34] revealed that municipal waste dumpsites harbor large diversity and complex communities of bacteria that has triggered further research on the public health implication of wastes in such man-made environment. As the result, recent researchers have given considerable attention to deeply understand the ecology of ABR in the natural environment, particularly on the factors aggravating the emergence of ABR bacteria. With this view, this study was initiated to contribute scientific information on the prevalence of ABR bacteria isolated from a solid waste dumpsite in Bahir Dar city.

In the present study, bacteria related to *Staphylococcus, Escherichia, Bacillus, Pseudomonas, Enterococcus, Klebsiella, Salmonella, Shigella, Proteus* and *Citrobacter* were detected in variable frequencies. Isolates related to *Staphylococcus, Escherichia, Bacillus Pseudomonas* and *Enterococcus* species were predominant bacteria. Oviasogie and Agbonlahor [35] also reported these bacteria as dominantly encountered species in solid waste dumpsites. The occurrence of members of the enteric and other human-associated bacteria from the dumping site was also reported in the previously conducted studies in Nigeria [20], Ghana [15] and Kenya [19]. Even, high-throughput sequencing of 16S rRNA Gene, a method that does remove culture-based anomaly, revealed that *Escherichia* spp. were found to be among the dominant bacteria in municipal dumpsite [34]. This is not a surprise as the solid waste dumpsites can usually be highly contaminated with human and animal feces in low-income countries.

Several other bacteria, which were not detected in this study, including *Vibrio cholerae, Enterobacter, Providencia, Yersinia, Morganella, Serratia* and *Acinetobacter* species were also isolated from the waste dumpsite soil [19, 20]. The difference in the type of bacteria recovered in this study and previous studies might be due to several factors, such as differences in the complexity of the dumped wastes, physicochemical parameters of the dumpsite soil, and geographical and seasonal factors in the study areas. That means the heterogeneity of the dumpsite environment could result in a heterogeneous population of soil bacteria [36].

Even though conclusive evidence is obtained from stain level identification, the bacteria isolated in the present study are more likely pathogenic, which may be attributed to the disposal of complex wastes in the municipal waste dumpsite that is originated from various sources in Bahir Dar city. Members of *Staphylococcus, Pseudomonas, Klebsiella, Citrobacter, Proteus*, and *Escherichia* were frequently reported earlier as potential pathogens from the dumping site [15, 18, 35].

Regarding isolates related to *Bacillus* spp., some other studies conducted in solid waste dumpsites reported *Bacillus* spp. as a predominant group among the isolated bacteria [15, 37]. The occurrence of *Bacillus* spp. in the dumpsite soil at a relatively higher proportion is likely due to its wide range of physiological capabilities, extracellular enzymes, the formation of highly resistant endospores to hostile physical and chemical conditions, and the production of metabolites having antagonistic effects on other microorganisms [38]. These capabilities enable the organism to grow in many environments and withstand harsh environmental conditions including polluted environments that exert selective pressure on the proliferation of specific groups of soil bacteria.

In this study, 10 antibiotics, represented from 8 antibiotic categories, were tested on the isolated bacteria. A high degree of ABR rate was recorded from Bahir Dar city MSWDS. The overall ABR rate recorded in this study is the highest (85.9%) compared to that of previous reports from non-clinical samples. Almost all of the isolates were 100% resistant to at least one antibiotic drug showing that ABR is distributed among the microbial communities in the area. This high ABR rate may also be contributed by the intrinsic resistance. However, it is recently speculated that the intrinsic resistance may serve as the origin of acquired resistance [39, 40].

Most isolates (73-100%) were resistant to amoxicillin, vancomycin and nalidixic acid, and sizable proportions of the isolates were also resistant to sulfonamide, tetracycline and streptomycin. Several previous studies also reported a high prevalence of ABR among bacterial isolates not only from clinical samples but also from non-clinical environments. For example, a study in Nigeria by Oviasogie et al. [35] reported similar observations that most of the bacterial isolates were intermediary resistant or resistant to tetracycline, nalidixic acid and sulfonamide. Contrary to the results of this study, a lower resistance rate to amoxicillin (33-69%) was reported by previous studies conducted in Ethiopia [3]. Similarly, most of Enterococcus (75%) and Staphylococcus (92%) were found to be resistant to vancomycin, which is far higher than the previously reported resistance rate of Enterococcus (8%) and Staphylococcus species (20%) to the same antibiotics [3]. The differences in the degree of resistance rates might be due to the difference in the sample source and other pertinent factors. It is to be noted that vancomycin has long been used to treat Gram-positive bacterial infections [41]. However, the presence of vancomycin resistant Staphylococcus spp. was undoubtedly reported by Smith et al. [42]. Regarding nalidixic acid, Zothanpuia et al [37] found out that 70% of the isolated bacteria isolated during their study were resistant to nalidixic acid, which is a comparable resistant rate recorded in the present study (73.3%).

On the other hand, most isolates were susceptible to erythromycin, ciprofloxacin, chloramphenicol and gentamicin. This is in line with the known fact that gentamicin is effective against a wide range of bacterial infections, mostly Gram-negative bacteria including Pseudomonas, Proteus, Escherichia, Klebsiella, Enterobacter, Serratia species as well as Gram-positive bacteria, such as Staphylococcus spp. Similarly, ciprofloxacin is known to be effective against most Gram-negative bacteria, such as E.coli [43] and Pseudomonas spp.[7], and considerable proportions of Gram-positive bacteria [44]. Almost all isolates related to Bacillus spp. were found to be susceptible to gentamicin (100%), chloramphenicol (100%), and ciprofloxacin (90%) which is in line with a study result by Banoon et al. [45].

In this study, a substantial prevalence of multidrug resistance (MDR) bacterial isolates was recorded from soil samples of Bahir Dar city MSWDS. The overall MDR rate was 49.3%, where all isolates, with the exception of isolates related to Bacillus spp., demonstrated multidrug resistance feature. A comprehensive review by Muhie et al. [46] on antibiotic use and resistance pattern in Ethiopia found a 59.7% pooled prevalence of MDR. However, almost all of the studies included in this review were clinical or hospital-based. Thus, our study documented the highest prevalence of MDR from non-clinical samples. High prevalence rates of MDR were recorded among isolates related to Escherichia (75%), Staphylococcus (68.8%) and Pseudomonas (62.5%), Klebsiella (60%) species, showing the public health risk that may be posed due to these bacteria. Lower resistance rates by Staphylococcus spp. were reported by previous studies. For example, a review by Deyno et al. [47] 2017 showed lower resistance to ciprofloxacin (19%), vancomycin (11%), tetracycline (62%) and to amoxicillin (77%) compared to shown in this study. Even, Ansari et al. [48] reported 100% susceptible Staphylococcus spp. isolated from clinical samples. Moreover, a 10 years study results showed an overall 45.38% drug resistance rate by Escherichia coli in Ethiopia [49], which is still lower than the resistance rate recorded in this study. The contribution of Pseudomonas spp. to the environmental resistome is also large [7], which is also shown in this study.

Conclusions

In this study, almost all isolated bacteria from Bahir Dar city municipal solid waste dumpsite were antibiotic-resistant (ABR), warning a high public health concern. Especially, isolates related to *Staphylococcus, Escherichia, Pseudomonas* and *Klebsiella* species, which demonstrated a high prevalence of MDR, call for special attention. Thus, the results of this study demonstrate that municipal solid waste can serve as a reservoir of ABR bacteria, and the poor waste management system in this area can be a serious health risk from drug-resistant pathogens. Erythromycin, ciprofloxacin, chloramphenicol and Gentamicin were the most effective antimicrobial agents against almost all the tested bacterial isolates, which is also a well-recognized fact. However, isolates exhibited a high rate of intermediary resistance and resistance features to amoxicillin, vancomycin and nalidixic acid, and a considerable rate of resistance to tetracycline, streptomycin and sulfonamide may present high public health risk. Moreover, the occurrence of isolates demonstrating multidrug resistance features at 49.3% prevalence gives serious implications from the public health point of view. Several other authors also revealed a high prevalence and distribution of ABR in waste dumpsites that could pose a risk of spreading the ABR to opportunistic pathogens [5, 37]. Detail studies to get insight into the isolates at strain levels and strengthening waste management system at household to community levels is highly recommended in order to reduce the public health risks imposed by drug resistance bacteria. Moreover, periodic monitoring using an antibiogram plays an important role in the reduction of municipal solid waste-driven antibiotic resistance.

List Of Abbreviations

ABR	Antimicrobial Resistance
AMX	Amoxicillin
C	Chloramphenicol
CIP	Ciprofloxacin
E	Erythromycin
G	Gentamicin
I	Intermediary resistant
MDR	Multidrug Resistance
MSWDS	Municipal Solid Waste Dumpsite
NA	Nalidixic acid
R	Resistant
ST	Streptomycin
SA	Sulfonamide
S	Sensitive/Susceptible
TE	Tetracycline
VA	Vancomycin

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests

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Tables

Table 1: Antimicrobial susceptibility profiles of bacterial isolates recovered from Bahir Dar city SWDS, Ethiopia, 2021.

Standard antibiotic discs tested	No. of isolates tested	No. of resistant isolates (%)	No. of intermediary resistant isolates (%)	No. of sensitive isolates (%)
AMX (25 µg)	30	30 (100)	0	0
VA (30 µg)	23	20 (87)	2(8.7)	1(4.35)
NA (30 µg)	60	44(73.3)	7(11.67)	9(15)
SA (250 µg)	24	12(50)	3(12.5)	9(37.5)
TE (30 µg)	68	33(48.5)	11(16.18)	24(35.29)
ST (30 µg)	22	12(54.5)	4(18.18)	6(27.27)
GN (10 µg)	63	4(6.3)	19(30.16)	40(63.49)
C (30 µg)	71	2(2.8)	14(19.72)	55(77.46)
CIP (5 µg)	71	2(2.8)	10(14.08)	59(83.1)
E (15 µg)	11	0(0)	1(9.09)	10(90.91)

Keys: AMX=amoxicillin; VA=vancomycin; NA= nalidixic acid; SA=sulfonamide; TE=tetracycline; ST=streptomycin; GN=gentamicin; C=chloramphenicol; CIP=ciprofloxacin; E=erythromycin

Table 2: The proportion of multidrug resistant bacterial isolates recovered from Bahir Dar city MSWDS, Ethiopia, 2021.

Isolates related to	Frequency (%)	Frequency of MDR isolates (%)	Frequency of resistance isolates at least to one antibiotic (%)
Staphylococcus spp.	16(22.5)	11(68.8)	14(87.5)
Escherichia spp.	12(16.9)	9(75)	12(100)
Pseudomonas spp.	8(11.3)	5(62.5)	8(100)
Klebsiella spp.	5(7)	3(60)	5(100)
Enterococcus spp.	8(11.3)	3(37.5)	8(100)
Salmonella spp.	6(8.5)	1(16.7)	6(100)
Bacillus spp.	10(14.1)	0	2(20)
Shigella spp.	2(2.8)	1(50)	2(100)
Proteus spp.	2(2.8)	1(50)	2(100)
Citrobacter spp.	2(2.8)	1(50)	2(100)
Total no. of isolates	71	35(49.3)	61(85.9)

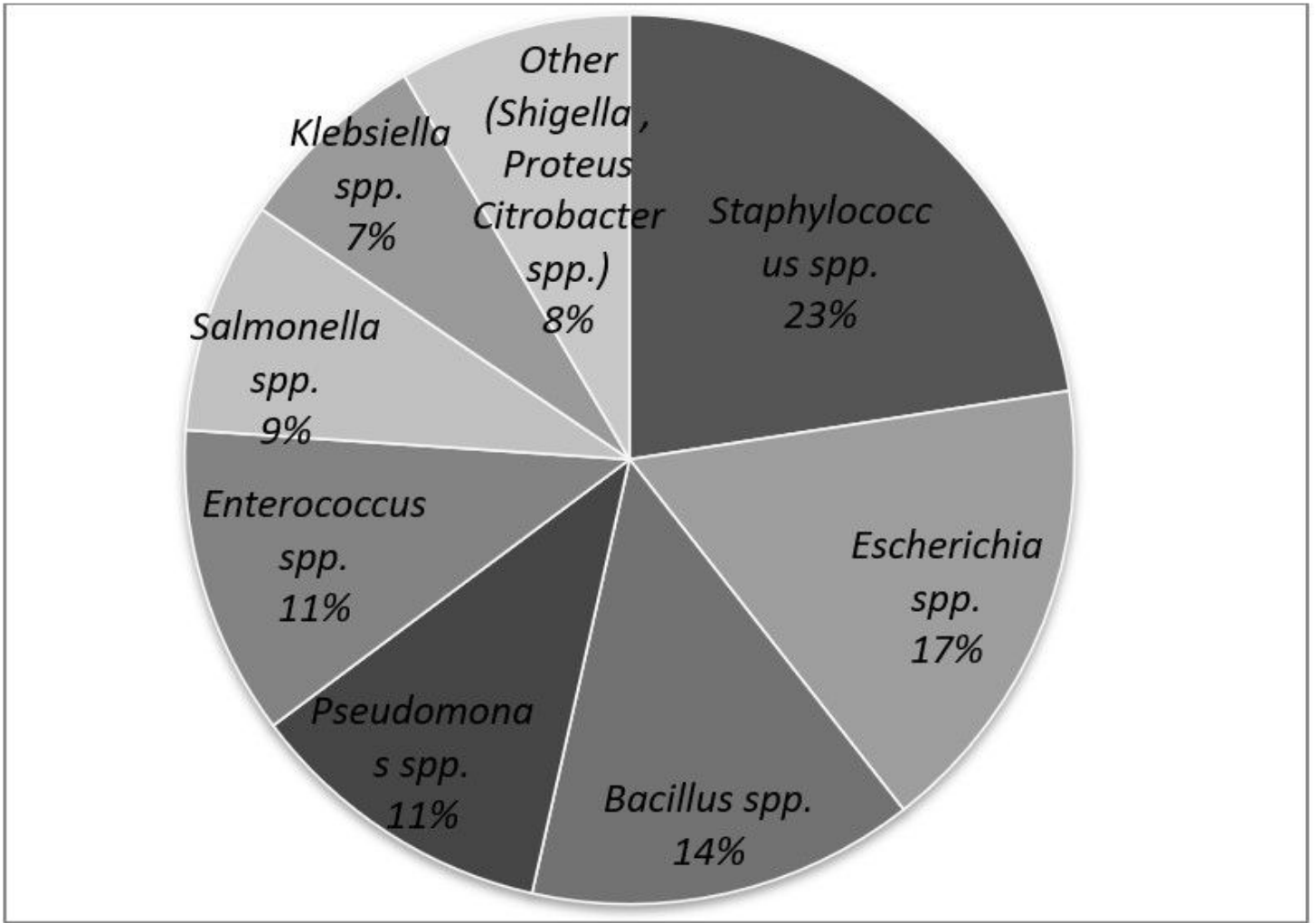Key: MDR= Multi Drug Resistance

Figures

Figure 1

The relative proportion of bacteria isolated from Bahir Dar city MSWDS, 2021

Supplementary Files

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