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Antibacterial metabolite prospecting from Actinomycetes isolated from waste damped soils from Thika, central part of Kenya

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ABSTRACT

Objective: To evaluate antibacterial activity of the metabolites produced by actinomycetes isolates isolated from waste damped soil in Thika.

Methods: Soil samples were collected randomly from selected waste damping sites and composite soil samples were prepared. Composite soil samples were pre-treated with dry heat and $CaCO_3$. 0.1 mL of soil suspension from 10^{-5} serial diluted composite soil sample spread on selective media for selective growth and isolation. The primary and secondary screenings and evaluation of antibacterial active isolates were done by streak plate and well diffusion assay, respectively.

Results: 29 (23.2%) isolates showed antibacterial activity during primary screening. From these, isolate KGT22 showed 30 ± 0 mm, 31.3 ± 0.6 mm, 30 ± 0 mm and 36 ± 1 mm inhibition zone against *E. coli* ATCC 25922, *S. boydii*, *S. typhi* and *V. cholerea*, respectively. Isolate KDO24 showed antibacterial activity against both MRSA (16.25 ± 0.50 mm) and *E. coli* (26.5 ± 0.58 mm). Supernatants from 11 (37.93%) isolates showed antibacterial activity during secondary screening. Supernatant from BML45, KGT31 and PLS 34 showed better inhibition zone (17 ± 1 mm) against *E. coli* ATCC25922, *S. boydii* and *S. typhi*, respectively.

Conclusions: Therefore, antibacterial activity result showed that soils collected from Thika waste damping sites are potential sources of antibacterial producing isolates.

1. Introduction

Actinomycetes (Actinobacteria) are unicellular Gram-positive prokaryotes, filamentous, aerobic bacteria having DNA with high G+C composition[1]. They are widely found in natural environments and widely distributed groups of soil microorganisms[2]. Garden rehizosphere soil[3,4], mangrove forest soil[5], virgin soil[6], agriculture field soil[7], acidic soil[8], compost soil[3] are just list of a few terrestrial places where actinomycetes are found.

Actinomycetes produce secondary metabolites (organic acids, enzymes, antibiotics or antimicrobial compounds) that are industrially, biotechnologically and environmentally valuable compounds. Previous studies have shown that antimicrobial compounds produced by actinomycetes have antiviral[9,10], antifungal and antibacterial[3,5,6] activity.

The current challenge in the treatment of infectious disease is the occurrence of drug resistant in both Gram-negative and Gram-positive bacteria pathogens worldwide. *Escherichia coli*, *Vibrio cholerae*, *Salmonella enterica*, *Enterobacter* spp. and *Staphylococcus aureus* are some of multi drug resistant developing bacteria[11]. Moreover, *Salmonella* species, *Shigella* species, *Vibrio* species and *E. coli* are frequently occurring drug resistant bacteria in East Africa region[12]. In this region, the status of antibacterial agent research against these antibacterial resistant bacteria is inadequate.

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In addition to this, majority of the ecological niches of this region still remain unexplored yet for the purpose of searching novel groups of actinomycetes for novel antibacterial discovery.

Thus, the need for effective antibacterial compounds from actinomycete isolated from potential soils that were not researched before is high priority. However, searching of antibacterial compound from actinomycetes isolated from waste damped soil from Thika area has not been investigated. Waste damping areas may be potential niches for actinomycetes due to availability of diverse nutrients. Thus, the main purpose of this study was to isolate actinomycetes from Thika waste dump sites and to determine antibacterial activity of them against selected bacteria pathogens using streak plate method (direct challenge of isolates against pathogens) and well diffusion assay using supernatants from the isolates.

2. Materials and methods

2.1. Study area and sampling sites description

The study area was carried out in Thika industrial and waste dumping areas, found in Thika district located in Kiambu County. Its location has 3°53' and 1°45' south latitudes and 36°35' and 37°25' east longitudes[13]. Thika is an industrial town, 42 km away from northeast of Nairobi, Kenya. Wastes from industries were damped temporally around the industry compound. Kiganjo (Kang'oki) waste damping area receives both the industrial and municipal wastes. The selected sampling sites for the present study were BIDCO Africa Ltd waste disposal site (BAL), BIDCO Africa Ltd waste damping site (BAD), Poly Sack Ltd waste damping site (PLS), Bakex Millers Ltd waste dumping site (BML), Kiganjo waste damping site one (KDO), Kiganjo waste damping site two (KDT), Kiganjo waste damping site three (KGT). The control site selected was Mangu' shrubs rehizosphere site (MRS). The geographical location of different soil sampling sites is described in Figure 1.

2.2. Composite soil preparation, analysis and pre-treatment

Nine plots were prepared per site in 16 m^2 area using GPS coordinate points as a reference point. Soil samples from each plot were collected aseptically from 0-20 cm depth[14] at depth of 0-5 cm, 5-10 cm, 10-15 cm[15]and 15-20 cm[16]. Four composite soil samples were prepared based on depth per site and the samples were packed using sterile polyethylene bag[5,15,16] to minimize soil moisture lose. From 8 sites 32 composite soil samples were prepared, packed and transported to Jomo Kenyata University of Agriculture and

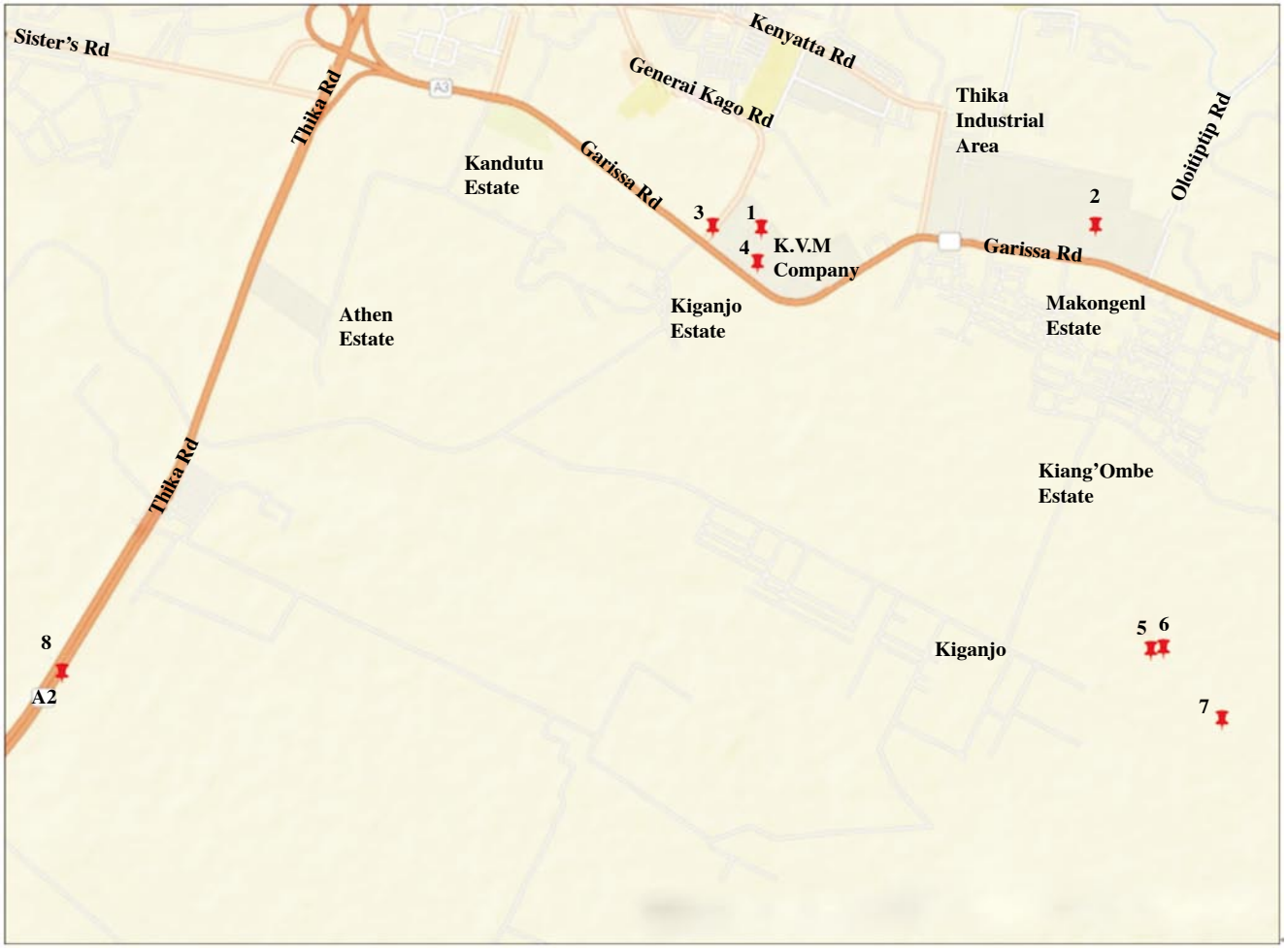

Figure 1. The Geographical location and representation of the soil sample sites in Thika district, Kenya (prepared by Temitope Idowu).
Key: 1 (BAL), 2 (PLS), 3 (BAD), 4 (BML), 5 (KDO), 6 (KDT), 7 (KGT) and 8 (MRS).

Technology (JKUAT), Zoology laboratory.

The moisture content and the pH value of the composite soil sample were analyzed according to George *et al*.[17] and Saha and Santra[18]. The composite soil samples were air dried in this lab at room temperature according to Azira *et al*.[5] and Messaoudi *et al*.[16]. The soil samples were transported to Kenya Industrial research Development and Innovation (KIRDI). Dry heat pre-treatment of composite soil sample was done according to Azira *et al*.[5] and chemical treatment using calcium carbonate or $CaCO_3$ (1%) was done according to Messaoudi *et al*.[16].

2.3. Isolation of actinomycetes from composite soil samples

Serial dilution and spread of soil suspension on starch casein agar plate was done according to Bizuye *et al*.[3], Sudha and Hemalatha[4], and Azira *et al*.[5] with minor modification. 10 gram of pre-treated composite soil samples were mixed with 90 mL sterilized saline water to make 10^{-1} stock dilution. Serial dilution was done up to 10^{-5} and 0.1 mL suspension from 10^{-5} was taken and spread on starch casein agar media supplemented with 100 μg/mL cyclohexamide and 1 μg/mL pencillin (experimental group). Experimental group and negative control (media without soil sample) in duplicates were incubated at 28 °C[19,20] for two weeks and the numbers of colonies were recorded. Different colonies per sample from the plates were subcultured in to starch casein media for growth and the recovered isolates were temporarily preserved at 4 °C for further use.

2.4. Bacterial pathogen suspension preparation and standardization

A total of six test (five Gram-negative and one Gram-positive) bacterial pathogens used for this study were obtained from Kenya Medical Research Institute (KEMRI). These include *Escherichia coli* ATCC25922, *Salmonella typhi*, *Shigella boydii*, *Vibrio cholerae*, extended spectrum beta-lactamase (ESBL) producing *E. coli* and Methicillin resistance *Staphylococcus aureus* (MRSA). Except *E. coli* ATCC25922, other test pathogens used for this study were clinical isolates. Test bacterial suspension preparation and standardization was done according to Ataee *et al*.[21]. The test bacterial pathogens were streaked on Muller Hinton agar plate and incubated at 37 °C for 24 h to get pure colonies. Two to three colonies were taken and put in to test tube containing 3 mL sterile water and mixed well. By adding a colony or adding sterile water, each bacterial pathogen suspension was standardized using McFarland standard (0.5%) to use for antibacterial activity screening.

2.5. Primary screening of antibacterial producing actinomycetes

The primary screening was done according to Bizuye *et al*.[3] and Azira *et al*.[5]with some modification. The recovered (125) isolates were subcultured in to starch casein broth for three days growth at 28 °C. Each 3 days old isolate was streaked on starch casein plate media horizontally and were incubated at 28 °C for 8 days with negative control (starch casein plate). *E. coli* ATCC25922, *S. typhi*, *S. boydii* and *V. cholerae* suspensions were streaked perpendicular to the grown actinomycete isolate on starch casein plate and incubated at 37 °C for 24 h.

2.6. Antibacterial activity screening of potential isolates against MRSA and ESBL E. coli by streak plate assay

29 isolates that were showed antibacterial activity during primary screening was also streaked on the plate containing starch casein agar and incubated for 8 days at 28 °C. After 8 days incubation, the 18 h old suspensions of clinical MRSA and ESBL *E. coli* was streaked perpendicular to each isolates and incubated for 24 h at 37 °C. The antibacterial activity was determined by measuring zone of inhibition.

2.7. Comparison of number of active isolates in relation to incubation period during secondary screening

Secondary screening was done by well diffusion assay using supernatants from starch casein broth cultured isolates that was taken at different day incubation. Isolates (29) showed antibacterial activity during primary screening were selected for secondary screening. Five millilitre of 3 days old seed culture from each active isolates was added in to a flask containing 50 mL sterile starch casein broth and incubated for 13 days at 28 °C. The supernatant from each isolate (experimental group) and starch casein broth (negative control) was collected from centrifuged (at 10000 r/min for 10 min) cultures taken at 8, 9, 10 and 13 days old. Each pathogen (*E. coli* ATCC25922, *S. typhi*, *S. boydii* and *V. cholerae*) was swabbed on a sterile Muller Hinton agar. Eighty micro-litres supernatant from isolates, streptomycin (positive control) and starch casein broth (negative control) was added on each well on Muller Hinton agar. The triplicates were done and incubated for 24 h at 37 °C. The zone of inhibition result in millimetre was recorded.

2.8. Data analysis

Data collected from the experiments were analyzed using descriptive analysis by excel and SPSS version 20 and the mean ± SD were calculated. The value of the moisture content, pH, total count of CFU/g of collected soil sample, the distributions of recovered isolates were analyzed using Microsoft excels. The mean ± SD value of inhibition zone of antibacterial activity against selected bacterial pathogens was compared. These comparisons were performed using One-way ANOVA ranked with Tukey's multiple range tests with descriptive analysis by SPSS version 20. The differences were tested on $P < 0.05$ (95% probability level) and all statistical values at $P < 0.05$ are statistically significantly different.

2.9. Ethical consideration

The locations for collection of composite soil samples were waste

disposal and damping sites that were not involved any endangered or protected species, so it was not needed strict and specific permission. The topology, land structure and the plant and animal diversity of the places were not affected by using appropriate sample collection procedure.

3. Results

3.1. Sampling site description, composite soil sample analysis and colony counting

BAL and BAD sampling site has sandy soil with oiled waste dumped and sandy soil with sewage sludge waste disposal area, respectively. BML and PLS sampling sites has loam soil with wheat husk and sandy soil with ashes come from burned oily waste from the industry, respectively. On the other hand, different types of wastes that were transported from different industries and from the town were dumped on Kiganjo (Kangoki) area. KDO, KDT and KGT sites were randomly selected soil sampling sites in this waste damping area. KDO site has dark grey sandy with paper bags, wigs and paper wastes damped on it. Sandy soils with charcoal and ashes are the description of KDT site. KGT site has grey sandy soil with oiled slugs, broken glasses, avocado peels damped on it. On the other hand, MRS site has sandy and sticky soil in the shrubs.

A total of 32 composite soil samples were collected from 8 soil sampling sites. As the result indicated, the moisture content, pH and number of CFU/g of composite soil were varied among eight sampling sites. The highest moisture content was recorded from KDT site (48.65 ± 7.26%). Acidic soil was from both BAD (5.74 ± 0.37) and BML (5.95 ± 0.29), while alkaline soil was from KDT site (9.16 ± 0.24). The highest average colony count (16.75 ± 7.32 × 10^6 CFU/g) was recorded from KDO site. However, no growth was observed from BAD site (Table 1).

3.2. Distribution of recovered isolates with respect to sites

A total of 125 isolates were recovered from 7 sites where the largest number of isolates were recovered from KDO [56 (45%)]. Except from BAL site, all other five sites had greater number of recovered isolates when compared to control (MRS) site. From a total of 125, 29 (23.2%) isolates showed antibacterial activity against more than one selected pathogens where all of active isolates were recovered from six [PLS, BML, KDO, KDT, KGT and MRS (control)] sites. The greater number of active isolates were recovered from both PLS [8 (28%)] and KDO [8 (28%)] sites when compared to other sites and control or MRS site [3 (10%)]. According to this experiment there was no active isolate recovered from BAL site. From this result, we can conclude that the numbers of active isolates were varied from site to site (Figure 2).

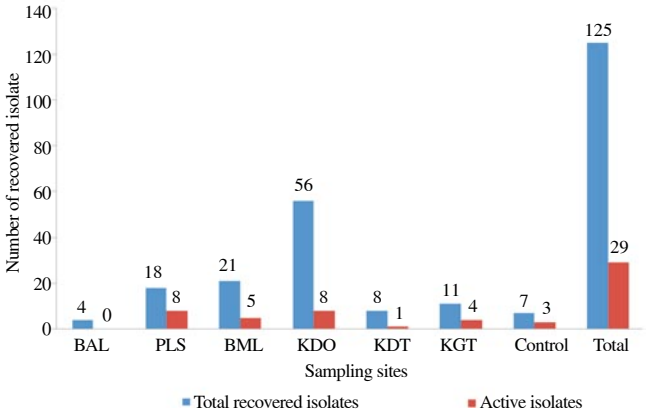

Figure 2. Distribution of isolates with respect to sampling site.

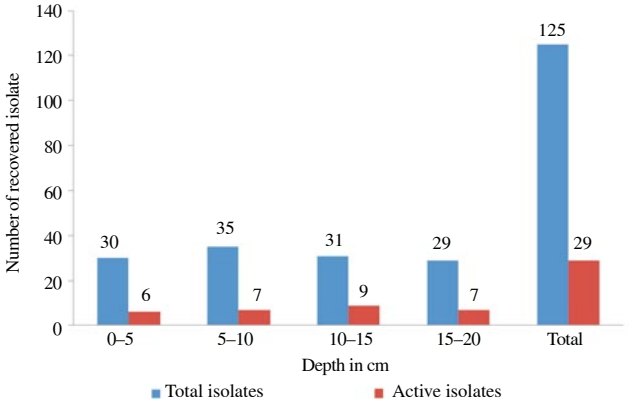

Figure 3. The distribution of recovered isolates with respect to depth.

3.3. Distribution of recovered isolates and active isolates with respect to sampling depth

From a total of 125 isolates recovered from 7 sampling sites at different depth, the greater number of isolates [35 (28%)] were recovered from 5–10 cm depth when compared to other depths.

Table 1
Sampling site description, soil sample analysis and number of CFU/g.

Sampling sites	Soil type with major dumped wastes	GPS coordinates (o)		MC (%)	pH	Average CFU/g (10^6)
		Latitude	Longitude			28 °C
BAL	Sandy with oiled sludge	-1.051134	37.086341	23.90 ± 1.88	8.12 ± 0.23	1.38 ± 0.95
PSL	Sandy with ashes	-1.050989	37.105572	16.44 ± 1.88	7.71 ± 0.20	1.88 ± 0.25
BAD	Sandy with sludge	-1.051035	37.083528	15.71 ± 6.17	5.74 ± 0.37	0.00 ± 0.00
BML	Loam with wheat husks	-1.053114	37.086125	14.33 ± 6.89	5.95 ± 0.29	3.00 ± 2.16
KDO	Sandy with wigs, paper bags	-1.075395	37.108762	33.23 ± 3.32	8.16 ± 0.15	16.75 ± 7.32
KDT	Sandy with charcoal	-1.075283	37.109494	48.65 ± 7.26	9.16 ± 0.24	1.63 ± 1.25
KGT	Sandy with sludge	-1.079382	37.112864	26.28 ± 3.06	7.70 ± 0.16	1.25 ± 0.50
MRS	Sandy and sticky	-1.076685	37.046068	22.13 ± 0.51	7.60 ± 0.29	1.50 ± 1.73
Starch casein media (Negative control)						0.00 ± 0.00

CFU/g: Colony forming unit per gram of soil, MC: Moisture content, o: Degree.

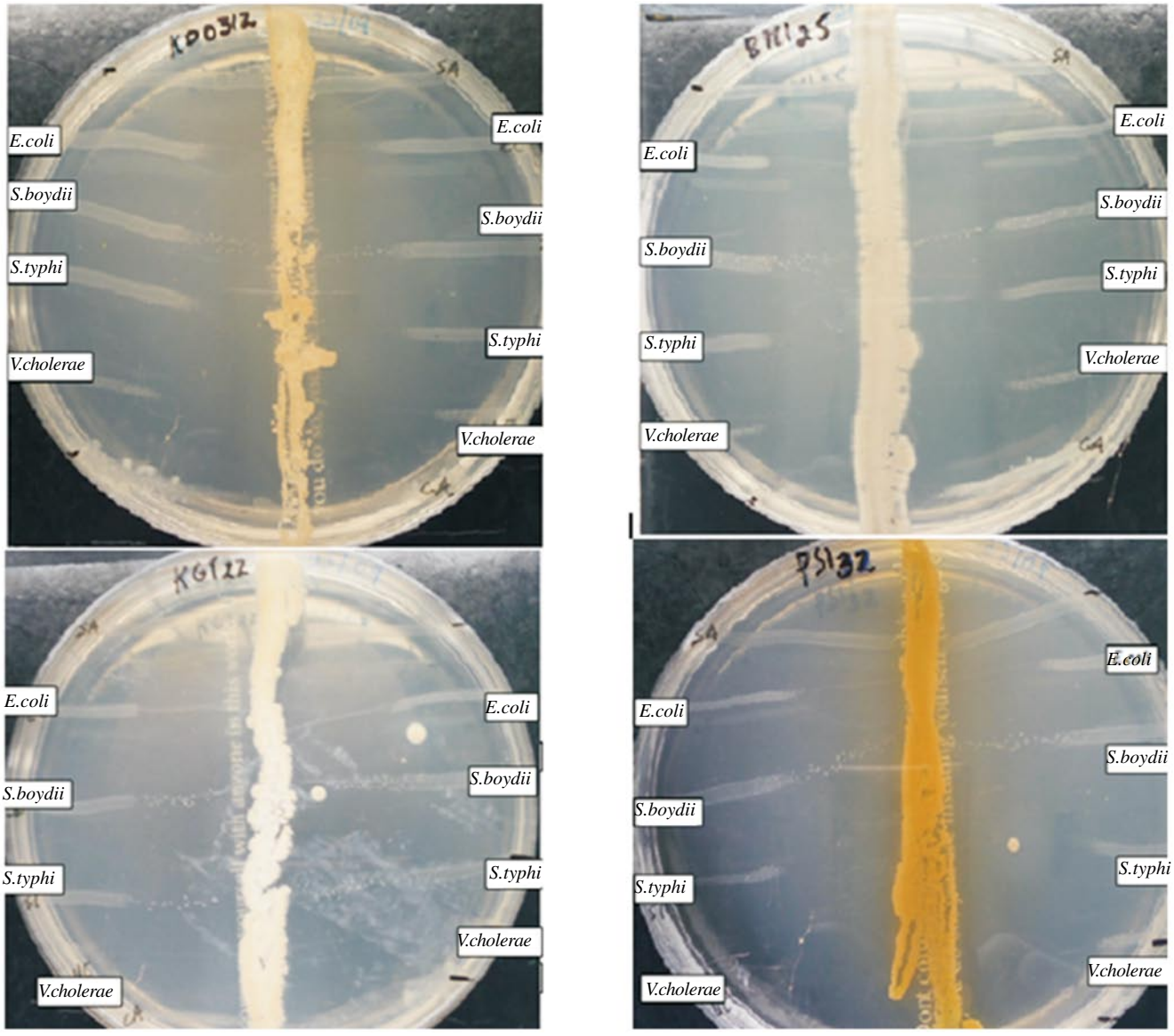

Figure 4. The inhibition zone of selected isolates (KDO123, KGT22, BML25 and PLS32) against selected pathogens.
Key: The clear distance between the isolate and the pathogen is the inhibition zone.

According to the result, the active isolates were found in all depth but the number varied from depth to depth. The greater number of active isolates [9 (31%)] were obtained from a depth of 10–15 cm when compared to other depths. From these result we can conclude that the active isolates were found from 0–20 cm depth range (Figure 3).

3.4. Comparison of bioactivity of isolates against selected pathogens during primary antibacterial screening

The antibacterial activity of active isolates against selected bacterial pathogens were compared and described using the inhibition zone measured in millimetre. The activity of selected isolates against selected pathogens using streak plate assay were depicted (Figure 4).

There was a significance difference ($P < 0.05$) of inhibition zone among the bioactivity of 29 active isolates against one or more selected bacteria. The highest bioactivity against *E. coli* ATCC25922 was recorded by KGT22 (30 ± 0.0 mm) when compared to MRS 44 (2.0 ± 0.0 mm) from control site. In addition, KGT22 active isolate also showed highest anti-*S. typhi* (30.0 ± 0.0 mm) and anti-*V. cholerae* (36.0 ± 1.0 mm) when compared to others. Moreover, *S. boydii* was more strongly inhibited by KGT22 (31.3 ± 0.6 mm) followed by BML44 (26.3 ± 0.6 mm), PLS11 (25.3 ± 0.6 mm) and PLS32 (25.0 ± 0.0mm (Table 2).

3.5. Antibacterial activity screening of potential isolates against MRSA and ESBL E. coli by streak plate assay

Antibacterial activity of 29 active isolates also tested against MRSA and *E. coli*. From these, only 5 (17.2%) isolates showed antibacterial activity against clinical MRSA and *E. coli* and the bioactivity showed significantly difference ($P < 0.05$) among active isolates. Isolate KDO24 showed highest inhibition zone against MRSA (16.25 ± 0.50 mm) and *E. coli* (26.5 ± 0.58 mm) when compared to others. Isolate KDO24 and KGT12 showed bioactivity against both MRSA and *E. coli* (Table 3).

Table 2
The antibacterial activity (mm) of isolates against bacterial pathogens during primary screening.

Sites	Active isolates	ATCC25922	S. boydii	S. typhi	V. cholera
	PLS11	22.3 ± 0.6^{k}	25.3 ± 0.6^{j}	26.0 ± 0.0^{op}	25.0 ± 0.0^{j}
PLS	PLS13	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	5.3 ± 0.6^{cde}	10.0 ± 0.0^{c}
	PLS23	16.0 ± 1.0^{ij}	15.7 ± 0.6^{h}	14.7 ± 0.6^{l}	20.0 ± 0.0^{h}
	PLS31	8.3 ± 0.6^{ef}	7.3 ± 0.6^{ef}	7.7 ± 0.6^{fg}	15.3 ± 0.6^{fg}
	PLS32	26.0 ± 1.0^{l}	25.0 ± 0.0^{j}	25.7 ± 0.6^{op}	32.0 ± 0.0^{l}
	PLS34	8.3 ± 0.6^{ef}	5.3 ± 0.6^{cd}	6.7 ± 0.6^{ef}	16.0 ± 1.0^{g}
	PLS41	8.3 ± 0.6^{ef}	5.0 ± 0.0^{c}	7.3 ± 0.6^{f}	19.0 ± 0.0^{h}
	PLS44	9.3 ± 0.6^{fg}	10.3 ± 0.6^{g}	11.0 ± 0.0^{ij}	12.7 ± 0.6^{d}
BML	BML22	10.3 ± 0.6^{g}	10.0 ± 0.0^{g}	11.7 ± 0.6^{ik}	12.3 ± 0.6^{de}
	BML25	22.0 ± 1.0^{k}	21.0 ± 1.0^{i}	20.3 ± 0.6^{m}	22.3 ± 0.6^{i}
	BML26	14.3 ± 0.6^{hi}	0.0 ± 0.0^{a}	15.0 ± 0.0^{l}	14.3 ± 0.6^{f}
	BML44	25.0 ± 0.0^{l}	26.3 ± 0.6^{j}	27.0 ± 0.0^{p}	30.3 ± 0.6^{k}
	BML45	7.3 ± 0.6^{de}	5.3 ± 0.6^{cd}	6.3 ± 0.6d^{ef}	15.7 ± 0.6^{fg}
KDO	KDO19	6.3 ± 0.6^{cd}	5.0 ± 0.0c	5.7 ± 0.6^{cde}	14.7 ± 0.6^{fg}
	KDO122	5.3 ± 0.6^{c}	7.0 ± 1.0^{de}	4.3 ± 0.6^{c}	10.0 ± 0.0^{c}
	KDO123	13.3 ± 0.6^{h}	20.0 ± 0.0^{i}	20.0 ± 0.0^{m}	30.0 ± 0.0^{k}
	KDO22	16.3 ± 0.6^{j}	20.3 ± 0.6^{i}	21.0 ± 0.0^{mn}	25.3 ± 0.6^{j}
	KDO24	10.3 ± 0.6^{g}	21.0 ± 1.0^{i}	22.0 ± 1.0^{n}	25.3 ± 0.6^{j}
	KDO38	8.3 ± 0.6^{ef}	7.7 ± 0.6^{ef}	9.0 ± 0.0^{gh}	15.3 ± 0.6^{fg}
	KDO310	5.3 ± 0.6^{c}	6.0 ± 0.0^{cde}	12.7 ± 0.6^{k}	20.0 ± 0.0^{h}
	KDO312	16.0 ± 1.0^{ij}	20.0 ± 0.0^{i}	25.0 ± 0.0^{o}	30.0 ± 0.0^{k}
KDT	KDT32	5.3 ± 0.6^{c}	6.3 ± 0.6^{cde}	10.0 ± 0.0^{hi}	11.3 ± 0.6^{cde}
KGT	KGT12	14.3 ± 0.6^{hi}	14.7 ± 0.6^{h}	15.0 ± 0.0^{l}	15.0 ± 0.0^{fg}
	KGT22	30.0 ± 0.0^{m}	31.3 ± 0.6^{k}	30.0 ± 0.0^{q}	36.0 ± 1.0^{m}
	KGT31	10.0 ± 0.0^{fg}	9.0 ± 0.0^{fg}	10.0 ± 0.0^{hi}	11.0 ± 0.0^{cd}
	KGT32	11.0 ± 0.0^{g}	10.3 ± 0.6^{g}	10.3 ± 0.6^{hij}	11.0 ± 1.0^{cd}
MRS (Control) site	MRS43	5.3 ± 0.6^{c}	6.3 ± 0.6^{cde}	5.0 ± 0.0^{cde}	15.0 ± 0.0^{fg}
	MRS44	2.0 ± 0.0^{b}	2.3 ± 0.6^{b}	0.0 ± 0.0^{a}	4.0 ± 0.0^{b}
	MRS45	3.0 ± 0.0^{b}	2.3 ± 0.6^{b}	2.0 ± 0.0^{b}	5.0 ± 0.0^{b}
Negative control		0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}

Table 3
The bioactivity of isolates against clinical MRSA and *E. coli* using streak plate method.

Isolates	MRSA	ESBL *E. coli*
PLS32	14.5 ± 0.58^{cd}	0.0 ± 0.0^{a}
BML25	13.75 ± 1.50^{c}	0.0 ± 0.0^{a}
BML44	8.0 ± 1.83^{b}	0.0 ± 0.0^{a}
KDO24	16.25 ± 0.50^{d}	26.5 ± 0.58^{c}
KGT12	10.5 ± 0.58^{b}	4.0 ± 0.82^{b}
Control	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}

Control: Media and clinical pathogen. Values are means ±SD. The outcomes not sharing a common superscript letter (a < b < c <…) in the same column are significantly different at $P < 0.05$.

3.6. Comparison of number of active isolates in relation to incubation period during secondary screening

The supernatants from 29 isolates were taken at different period of incubation and tested antibacterial activity against selected pathogens. Here only active isolates, susceptible pathogens and the antibacterial producing incubation period were described. From a total of 29 active isolates, supernatants taken from 8–13 days old culture of 11 (37.93%) isolates showed antibacterial activity against one or more selected pathogens. As antibacterial activity of the supernatant taken from 8 days old culture showed the highest number of isolates were active against *E. coli* ATCC25922 [11 (37.9%)] and *S. typhi* [6 (20.7%)] when compared to others incubation period. However, at 9 day incubation period, the highest number of active isolates against *S. boydii* [6 (20.7%)] were recorded when compared to other incubation periods (Figure 5). These results indicate that the number of incubation days influence the antibacterial activity of the supernatant.

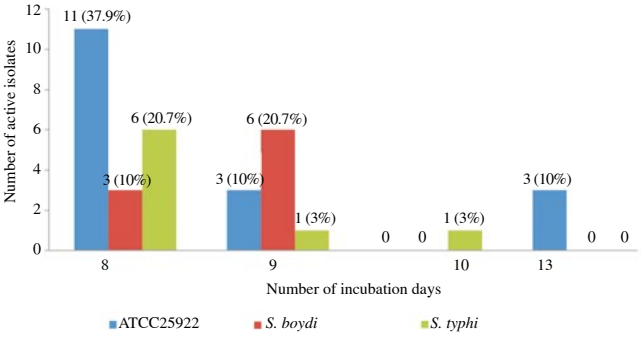

Figure 5. Comparison of the number of active isolates against selected pathogens with respect to incubation period.

3.7. Comparison of antibacterial activity of supernatants from selected incubation period of isolates during secondary screening

The antibacterial activities of the supernatant from 29 isolates were tested against selected pathogens by well diffusion assay. The antibacterial active isolates against one or more pathogens and susceptible pathogens are only described on Table 4 and Figure 6.

Table 4
Comparison of zone of inhibition of supernatant from selected incubation period.

Isolates	*E. coli* ATCC25922	*S. boydii*	*S. typhi*
	At 8 day	At 9 day	At 8 day
PLS13	15 ± 1^{bcd}	0 ± 0^{a}	0 ± 0^{a}
PLS31	16 ± 1^{cd}	0 ± 0^{a}	14 ± 1^{bc}
PLS34	15 ± 1^{bcd}	0 ± 0^{a}	17 ± 1^{d}
PLS41	16 ± 1^{cd}	0 ± 0^{a}	16 ± 1^{cd}
PLS44	14 ± 0^{bc}	0 ± 0^{a}	17 ± 1^{d}
BML45	17 ± 1^{d}	15 ± 0^{b}	0 ± 0^{a}
KDO19	17 ± 1^{d}	16 ± 1^{bc}	0 ± 0^{a}
KDO38	17 ± 0^{d}	15 ± 1^{b}	0 ± 0^{a}
KDT32	15 ± 1^{bcd}	15 ± 0^{b}	0 ± 0^{a}
KGT31	13 ± 1^{b}	17 ± 1^{c}	12 ± 1^{b}
KGT32	15 ± 1^{bcd}	17 ± 0^{c}	13 ± 1^{b}
STP	20.3 ± 0.6^{e}	24 ± 1^{d}	24.7 ± 0.6^{e}
Broth	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}

STP: Streptomycin or positive control, Broth: Negative control, 0: No activity observed. Values are means ± SD. The outcomes not sharing a common superscript letter (a < b < c < d…) in the same column are significantly different at $P < 0.05$.

The antibacterial activity of the supernatants from 11 (37.93%) isolates showed significantly difference ($P < 0.05$) between them and the control. The antibacterial activity of supernatants from isolate BML45 (17 ± 1 mm), KDO19 (17 ± 1 mm) and KDO38 (17 ± 0 mm) showed greater inhibition against *E. coli* ATCC25922 when compared to supernatants from other isolates but smaller than when compared to streptomycin (20.3 ± 0.6 mm). The greater inhibition zone against *S. boydii* was recorded by supernatants from both isolate KGT31 (17 ± 1 mm) and KGT32 (17 ± 0 mm) when compared to other supernatant activity but smaller than streptomycin

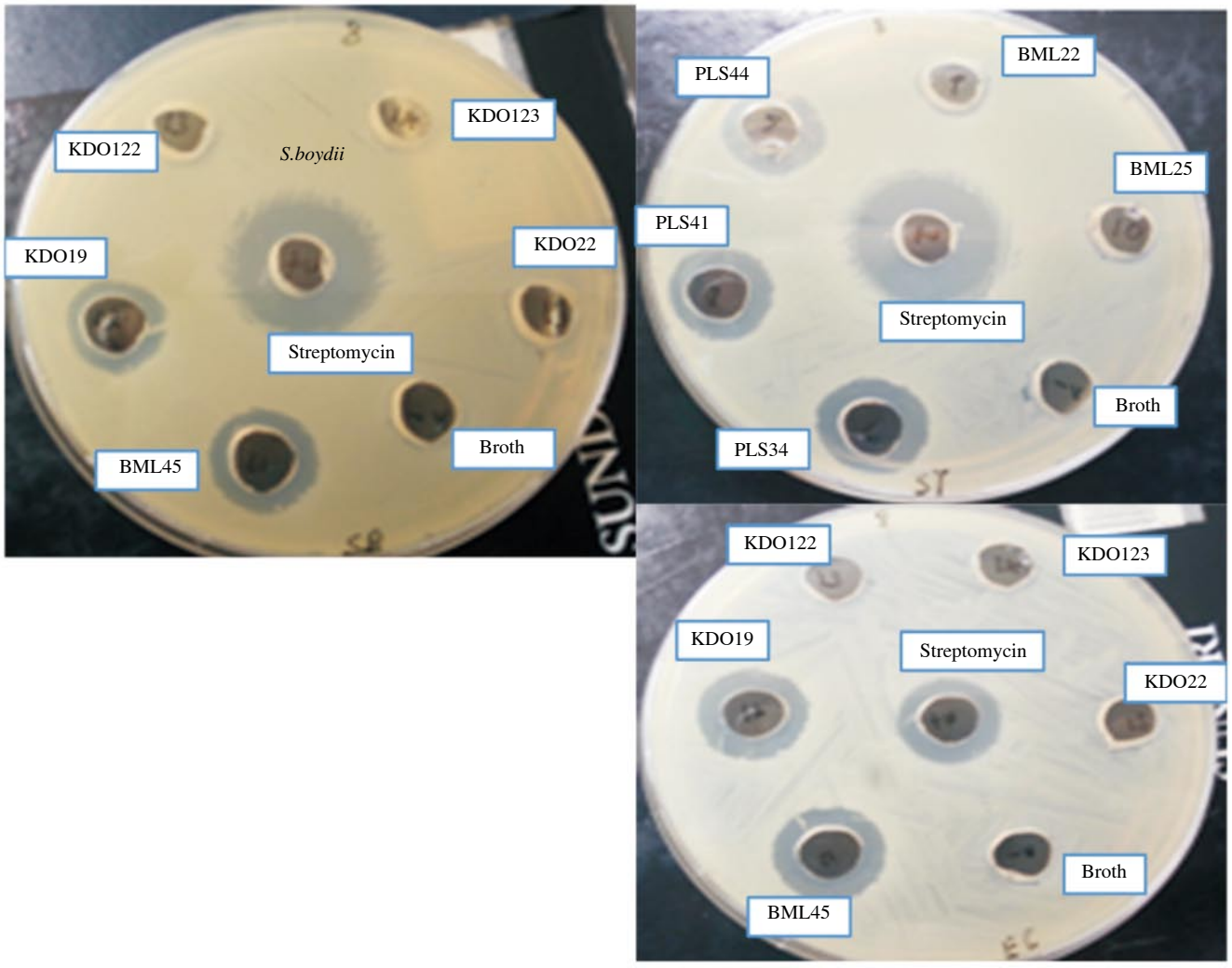

Figure 6. The inhibition zone by supernatant from 8 day cultured isolates against selected bacteria pathogens.

(23.7 ± 0.6 mm). The antibacterial activity of supernatant from isolate PLS34 (17 ± 1 mm) and PLS44 (17 ± 1 mm) showed the highest activity against *S. typhi* when compared to other supernatant activity but smaller than when compared to streptomycin (24.7 ± 0.6) (Table 4).

4. Discussion

The emergency of antibacterial resistant bacteria is a cause for concern. It is the basic challenge for effective treatment of infectious disease throughout the globe including the continent of Africa. *E. coli*, *Salmonella* sp., *Shigella* sp. and *Vibrio* sp. are some of frequently occurring drug resistant groups of Gram-negative bacteria in East Africa region[12]. Thus, searching of effective antibacterial product from actinomycetes isolated from unexplored environments of soil is required. Identifying and selecting such soil sampling areas in local districts may lead to getting of such biotechnologically and pharmaceutically valuable producing isolates. Thika waste damping sites are unexplored area for these purposes.

The present study showed that 23.2% antibacterial active isolates were recorded in Thika waste damped sites. This indicated that the frequency of getting antibacterial isolates in the present study area was greater than 16.6%[3] and smaller than when compared to 36.4%[6] and 55.7 %[5] finding. This variation occurred may be due to difference in geographical location of the sampling sites and sample pre-treatment methods. However, from present study 23.2% isolates showed either anti-*E. coli* ATCC25922, *S. boydii*, *S. typhi* and or *V. cholerae* activity which was better than when compared to none of them showed anti-*E. coli* ATCC25922[6], 12.8% of active isolates showed anti-*E. coli* ATCC25922[5] and 16.6% isolates showed anti-*E. coli* and anti-*S. typhi* ATCC9289 activity[3]. Our study made difference by indicating potential waste damping sites for isolation of antibacterial producing isolates and contributing more biologically active isolates in this study area. Therefore, the present study fills the research gap on searching of antibacterial producing isolates in these particular areas against Gram-negative bacteria pathogens.

The present study showed that some of isolates were showed good antibacterial activity against these pathogens and others were not. The antibacterial activity against *E. coli* (26.5 ± 0.58 mm), *S. boydii* (31.3 ± 0.6), *S. typhi* (30 ± 0 mm), *V. cholerae* (36 ± 1 mm) and MRSA (16.25 ± 0.5 mm) was best results recorded from the present study. As the previous study showed that the highest antibacterial activity was 30 ± 2 mm against *E. coli* ATCC25922 and 32 ± 2

mm against *S. typhi* ATCC9289[3] and > 30 mm against *E. coli* ATCC25922[5] was recorded which was greater than the present finding (26 ± 1.0 mm) against *E. coli* ATCC25922. These differences may be due to diverse types of active isolates isolated from different natural environments.

According to the present result, Thika industrial waste damping soils have potential isolates that show effective antibacterial activity against selected pathogens. And the number of active isolates that showed antibacterial activity varies from site to site. Thus, this study confirmed that soil samples collected from waste damping sites in Thika have a potential antibacterial producing isolates. However, further research is under progress for functional and chemical characterization of antibacterial compounds and synthesis of antibacterial silver nanoparticle using metabolites from potential isolates as well as identification of selected isolates at species level.

Conflict of interest statement

We declare that we have no conflict of interest.

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