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Bacillus Species Found Antagonistic against Bacteria Isolated from Currency Notes in Local Circulation

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Abstract
Bacterial species from currency notes and coins obtained from people belonging to different professions of Lahore, Pakistan were evaluated for their antagonistic activity against bacillus species isolated from the same source. A total of twenty nine isolates were identified based on morphology, growth on selective and differential media and biochemical tests. Spot-on-the-lawn deferred antagonism method was used to observe the antagonistic activity of the bacillus sp. isolates. It was found that six bacillus sp. isolates [*Bacillus cereus* (B.c), *Bacillus subtilis* (B.s), *Bacillus mycoides* (B.m), *Bacillus licheniformis* (B.l), *Bacillus vallismortis* (B.v) and *Bacillus thuringiensis* (B.t)] showed antagonism against the other bacterial isolates. Three isolates viz., B.c, B.s and B.l showed broad spectrum of antagonistic activity (+++), B.s, B.l, B.m and B.t showed antagonistic activity (+) while B.s, B.c, B.m, B.v, B.l and B.t showed antagonistic activity. This study concluded that the Bacillus species have the ability to produce antimicrobial compounds which may be used to control microbial infections.
Keywords: Bacillus, antibiotic, antagonistic activity, deferred antagonism, zones of inhibition, money.

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Introduction

The exchange of money on a wide range of goods and services in countries all over the world is a very vector for transmission of diseases [1, 2] and this is the reason that paper currency trading from one individual to another is likely to spread microorganisms. If pathogenic bacteria contaminate these currencies, the incidence and mortality of these infectious agents is a serious matter to consider [3, 4]
Microorganisms commonly associated with banknotes include *Staphylococcus aureus*, α-haemolytic Streptococcus, Bacillus spp (its spores may stay attached for many years), *Escherichia coli*, Acetobacter spp, Enterobacter spp, Pseudomonas spp, Salmonella spp, viruses, fungi, eggs & larvae of worms, helminthes and parasites. Some banknotes associated bacteria are pathogenic, while others may cause opportunistic infections and may be a common cause of food poisoning [5]. *Staphylococcus epidermidis* is part of the normal human flora, usually the skin flora, and less commonly the mucosal flora [6]. Bacillus species are Gram positive facultative anaerobic or aerobic, sporulating rod shaped bacteria that spread widely in nature [7, 8], of being involved in food poisoning [9]. Bacillus species exhibit a wide range of physiological capabilities that allow the organism to thrive in every environment and compete favorably with other organisms in the environment due to their capacity to form spores produce metabolites that are cold, heat, desiccation and radiation stable having antagonistic influence on other microorganisms [10]. Bacillus species that produce antibiotics are *B. subtilis*, *B. licheniformis*, *B. brevis*, *B. polymyxa*, *B. circulans* and *B. cereus*. Polypeptide antibiotics produced by Bacillus that are used in medical treatments are gramycidin, bacitracin, tyrotricidin and polymyxin [11].
Bacilli are identified to yield more than 45 antimicrobial molecules and some of which are of clinical importance. Bacillus species produce antibiotics in soluble protein form that synthesize and secrete into the growing medium. So antibiotics they produce have been found to be cheaper and effective thus preferably in commercial production [12, 13].
Mostly Bacillus species are of remarkable significance because they produce antibiotics [14]. The capability of Bacillus species to synthesize a wide range of metabolites with antimicrobial activity in medicine and pharmaceutical industry, one of its potential is to control different kind of diseases in human, animals and plants when applied as a biological control agent [13]. In recent years, many

investigations have exploited the antimicrobial properties of Bacillus strains [15, 16, 17, 18, 19]. The purpose of this study was to observe the spot-on-lawn method to determine the most reliable way to detect the antagonistic activity and show the effect of antimicrobial agents inhibitory activity of bacillus species against certain Gram negative and Gram positive bacteria.

Materials and Methods

1.1 Bacterial strains

Twenty nine bacterial species, isolated from currency notes and coins, were used in this study. Morphological characterization of the cultures was done on microscopy, Gram staining, spore, capsule staining, growth on selective and differential agar media such as Mannitol Salt (MS), Eosin Methylene Blue (EMB), Salmonella Shigella (SS), Polymyxin Pyruvate Egg Yolk Mannitol Bromothymol Blue Agar Base (PEMBA), Pseudomonas Cetrimide, Blood, CLED, chocolate, brain heart infusion and MacConkey from pure culture and biochemical tests such as Catalase, Oxidase, Coagulase, Indole production, Methyl red, Voges-Proskauer, Citrate utilization, Urease (IMViC), Hydrogen Sulfide (H_2S), Triple Sugar Iron (TSI), Nitrate Reduction, Litmus milk reactions and Casein, Starch, Lipid and Gelatin hydrolysis tests were done [20].

1.2 Test-cultures

In all twenty nine test organisms were obtained from currency notes and coins *S. aureus, S. epidermidis, S. saprophyticus, S. pneumoniae, E. faecalis, S. viridians, S. pyogenes, M. luteus, M. nishinomiyaensis, M. agilis, M. roseus, B. subtilis, B. cereus, B. vallismortis, B. licheniformis, B. mycoides, A. salmonicida, E. coli, E. aerogenes, S. sonnei,* Shigella species, *K. pneumonia, K. oxytoca, P. aeruginosa, P. putida, S. enterica,* MRSA, *V. cholerae* and *S. marcesens*. Bacterial stock cultures were maintained at -20°C in 40% glycerol stock. During this study it was observed that bacillus species inhibited the growth of other bacterial species.

1.3 Antagonistic activity of Bacillus species against pathogens

Bacterial isolates were studied for antibacterial activity on nutrient agar by spot on the lawn deferred antagonism method [21, 22]. Antibacterial activity was checked on nutrient agar. The bacterial cultures were grown overnight at 37°C in incubator and mixed with physiological saline to match the turbidity to a 0.5 McFarland turbidity standard. Using sterile swabs, 1ml bacterial culture was spread over the nutrient agar plate and loop full culture of six bacillus species were spotted on inoculated plate equal distance apart and incubated at 37°C for 24 hours. Antibacterial activity was measured by the appearance of zone of inhibition around the culture and result was recorded in– ve= (0mm), + = (1-10mm), ++= (11-20mm), +++ = (21-30mm) [23]. The strains which scored positive were then assessed as [23, 24].

No inhibition: - (Bacterial growth was similar to that of control)

Weak inhibition: + (Bacterial growth was slightly inhibited by bacteria)

Average inhibition: ++ (Loosely arranged Bacterial growth over the bacterial zone)

Strong inhibition: +++ (Bacterial growth was completely inhibited before the bacterial zone).

Results and Discussion

The bacillus species such as B.c, B.s, B.m, B.l, B.t, B.v used to test the antagonistic activity against all bacterial isolates that were obtained from currency notes and coins samples.

Bacteria at different phases during growth produce primary and secondary metabolites. This is important not only in microbiology and biotechnology research but also gaining significance in commercial, industrial and agricultural purposes [22]. Production of antibiotic is a biological advantage of several types of soil fungi and bacteria can be a survival mechanism where organisms can eliminate competition and colonize a niche [13]. This ability can be explored in a variety of bacterial populations especially in those famous. Detection of new antibiotics from such natural and alternative sources are becoming increasingly important for the pharmaceutical industry [25] and pathogenic bacteria have become remarkably resistant to therapeutic agents commonly used [26].

Al-Ajlani et al., [8] in his research revealed the production of antibacterial substances and described that the bacitracin produced by it inhibits *E. coli* and *S. aureus*. Bacillus spp. isolated from another environmental source such as currency notes and coins possessed antagonistic activity against Gram positive and Gram negative similar in finding to Al-Ajlani study.

Seven *Bacillus* species were categorized for their antagonistic activity against *Staphylococcus, Shigella* and *Salmonella* pathogens. Three bacillus species

showed obvious antagonistic activity against broad spectrum of pathogenic isolates including multi

Table 1: Determination of the antagonistic activity of Bacillus species on bacterial isolates

Sr. #	Bacterial isolates	*B. c*	*B. s*	*B. m*	*B. l*	*B. t*	*B. v*
1	*S. aureus*	+	−	+	−	−	−
2	*S. epidermidis*	−	−	−	++	−	−
3	*S. saprophyticus*	+	+	+	−	+	−
4	*S. pneumonia*	−	−	−	−	−	−
5	*E. faecalis*	−	−	−	−	−	−
6	*S. viridans*	−	+++	++	++		−
7	*S. pyogenes*	−	−	+	−	−	−
8	*M. luteus*	+	−	−	−	−	+
9	*M. nishinomiyaensis*	+	+	−	+	−	−
10	*M. agilis*	−	−	−	−	−	−
11	*M. roseus*	−	++	−	−	−	−
12	*B. cereus*	+	+	++	−	−	+
13	*B. subtilis*	−	−	+	+++	−	−
14	*B. vallismortis*	−	−	−	−	−	−
15	*B. licheniformis*	−	+	−	−	+	+
16	*B. mycoides*	+	+	−	+	−	+
17	*A. salmonicida*	−	−	−	+	−	+
18	*E. coli*	−	+++	−	−	−	−
19	*E. aerogenes*	−	−	−	−	−	−
20	*S. sonnei*	−	−	−	−	−	−
21	*Shigella* spp.	+++	−	+	−	−	+
22	*K. pneumoniae*	+	−	−	++	−	−
23	*K. oxytoca*	−	++	−	−	−	−
24	*P. aeruginosa*	+	−	−	−	++	−
25	*P. putida*	−	−	−	−	−	−
26	*S. enterica*	−	+	−	−	−	−
27	MRSA	−	+++	−	−	−	−
28	*V. cholerae*	−	−	−	+	−	+
29	*S. marcesens*	−	−	−	+++	+	−

Key: B.c- Bacillus cereus; B.s- Bacillus subtilis; B.m- Bacillus mycoides; B.l- Bacillus licheniformis; B.t- Bacillus thuriengiensis; B.v- Bacillus vallismortis

In += (1mm-10mm) zone range, B.c showed inhibition, against S. aureus, S. saprophyticus, M. luteus, M. nishinomiyaensis, B. cereus, B. mycoides, K. pneumoniae and P. aeruginosa. B. s showed inhibition, against S. saprophyticus, M. nishinomiyaensis, B. cereus, B. licheniformis, B. mycoides and S. enterica. B. m showed inhibition, against S. aureus, S. saprophyticus, S. pyogenes, B. subtilis and Shigella species. B. l showed inhibition, against M. nishinomiyaensis, B. mycoides, A. salmonicida and V. cholera. B. t showed inhibition, against S. saprophyticus, B. licheniformis, S. marcescens and B. v showed inhibition, against M. luteus, B. cereus, B. licheniformis, B. mycoides, A. salmonicida, Shigella species and V. cholera.

In ++= (11mm-20mm) zone range, B.c and B.v showed no inhibition against any bacterial isolates, B.s showed inhibition, against M. roseus and K. oxytoca, B. m showed inhibition, against S. viridans and B. cereus, B. l showed inhibition against S. epidermidis, S. viridans and K. pneumonia while B. t showed inhibition against P. aeruginosa.

In +++= (20mm-30mm) zone range, B.c showed inhibition, against Shigella spp., B. s showed against S. viridans, E. coli, MRSA, B. l showed inhibition against B. subtilis and S. marcescens while B.m, B.t and B.v did not show any inhibition.

resistant species. Inhibitory effect on MRSA and *S. aureus* was due to the production of biosurfactant, identified for two *B. subtilis* strains [28]. In this study, Bacillus strains such as *B. cereus* and *B. subtilis* showed activity against *S. auerus*; *B. licheniformis* showed activity against *S. epidermidis* and *B. cereus, B. subtilis, B. mycoides, B. thuringiensis* showed activity against *S. saprophyticus. B. subtilis* showed activity against *S. enterica. B. cereus, B. mycoides, B. vallismortis* showed activity against *Shigella spp. B. subtilis* showed activity against *MRSA.* These results are almost similar to Moore study.

Antibacterial activity of all bacterial strains was examined by the preliminary screening test for bacteriocins i.e. “Deferred antagonistic assay” (lawn

of a sensitive indicator strain inoculated with test culture [29]. Eight strains showed antagonistic activity against sensitive strains (*B. subtilis, B. polymxa, E. coli, K. oxytoca,* and *S. aureus*) [22].

Different strains of *Bacillus* species showed antimicrobial activity against *S. aureus, M. luteus, P. fluorescens, P. aeruginosa, E. coli, Y. enterocolitica, B. megaterium, B. subtilis, B. thuringiensis,* MRSA, *M. flavus* and *C. albicans* [30, 31, 32]. Antibacterial activity methods showed that the *S. aureus* was the most sensitive indicator bacteria. The *Bacillus* strains were active mostly against Gram-positive but not Gram negative bacteria, although the *E. coli* was the most common resistant bacterium [32].

In this study *B. cereus* showed inhibition against *S. aureus, S. saprophyticus, M. luteus, M. nishinomiyaensis, B. mycoides, B. cereus, K. pneumoniae* and *P. aeruginosa. B. subtilis* showed inhibition against *S. saprophyticus, M. nishinomiyaensis, B. cereus, B. licheniformis, B. mycoides* and *S. enterica. B. mycoides* showed inhibition against *S. aureus, S. saprophyticus, S. pyogenes, B. subtilis* and *Shigella* species. *B. licheniformis* showed inhibition, against *M. nishinomiyaensis, B. mycoides, A. salmonicida* and *V. cholera. B. thuringiensis* showed inhibition, against *S. saprophyticus, B. licheniformis, S. marcescens* and *B. vallismortis* showed inhibition, against *M. luteus, B. cereus, B. licheniformis, B. mycoides, A. salmonicida, Shigella* species and *V. cholera.*

Bacillus spp. was identified as *B. sphaericus, B. circulans, B. megaterium, B. brevis, B. subtilis, B. licheniformis, B. cereus* and *B. coagulans*. Five Bacillus isolates show antimicrobial activity but other bacterial isolates did not show antimicrobial activity. The inhibitory effect of bacillus isolates was found against *P. fluorescens, S. aureus, B. megaterium, B. thuringiensis, M. flavus* and *B. cereus* [11].

Perez *et al.*, [33, 34] reported that *B. subtilis* showed antimicrobial activity against *E. coli, P. aeruginosa* and *M. luteus*. Aslim *et al.*, [30] found that bacterial isolates *B. subtilis B. thuringiensis* and *B. megaterium* were active against *E. coli* and *Y. enterocolitica*. This study showed that the bacterial isolates used have no inhibitory effects regarding *B. subtilis, E. coli, P. aeruginosa, M. luteus* and *Y. enterocolitica*. The different strains are reported as effective against *B. megaterium* [30], *B. subtilis* and *S. aureus* [34], Bacillus spp [35].

B. brevis was found to have antimicrobial activity against *S. aureus, B. thuringiensis*; *B. cereus* has better inhibitory effect against *B. thuringiensis, M. flavus, P. fluorescens* and *B. thuringiensis*. It is also found that isolates have much better inhibitory effects against the test bacteria in contrast to some antibiotics [11].

Further study, antimicrobial compounds produced by Bacillus strains which would lead to a better understanding of the mechanisms of antagonistic activity from bacillus and selection of new strains promising for use in the field of pharmacology and biotechnology.

Conclusion

Currency notes and coins samples from different professions were evaluated for isolating bacterial Gram positive and Gram negative isolates. Results showed that these currency notes and coins could be good source for isolating antibiotic producing Bacillus. Further investigations are necessary to identify and purify the active novel metabolites from these isolates that are still unknown.

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