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Original Article

Detection of microorganisms with antibacterial activities from different industrial wastes and GC-MS analysis of crude microbial extract

Detecção de microrganismos com atividades antibacterianas de diferentes resíduos industriais e análise por GC-MS do extrato microbiano bruto

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Abstract

Many soil microorganisms' i.e., bacteria and fungi produce secondary metabolites called antibiotics. These are used for the treatment of some of the bacterial, fungal and protozoal diseases of humans. There is a need for isolation of a broad spectrum of antibiotics from microorganisms due to the emergence of antibiotic resistance. In the present study two antibiotic producing bacteria *Klebsiella pneumoniae* and *Bacillus cereus* were isolated from pharmaceutical and poultry feed industry of Hattar, Haripur Pakistan. Total 10 waste samples were collected from different industries (Marble, Ghee, Soap, Mineral, Steel, Poultry Feed, Pharmaceutical, Qarshi, Cosmetic and Glass). Thirty-three bacterial strains were isolated from industrial wastes of these ten different industries. Fourteen out of thirty-three bacterial strains exhibited antimicrobial activities against at least one of the test microbes considered in this study including *Escherchia coli*, *Staphylococcus aureus* and *Salmonella typhi*. The bacteria were isolated by standard serial dilution spread plate technique. Morphological characterization of the isolates was done by Gram staining. Nine bacterial isolates out of fourteen were initially identified as *B. cereus* and five as *K. pneumoniae* through biochemical characterization. The antibacterial activities were tested by well diffusion method. Maximum number of antibiotic producing bacteria were isolated from pharmaceutical and poultry feed industry based on the results of primary screening, the most potential isolates S9, S19, S20, S22 and S23 were selected for secondary screening. The maximum activity against *E. coli* and *S. aureus* was recorded by bacterial isolate S19 i.e zones of inhibition of 6.5mm and 9mm while S20 showed 7.5mm and 6mm zones respectively. Molecular identification was carried out on the basis of 16S rRNA sequence analysis. Finally, the isolates were identified as *B. cereus* accession number LC538271and *K. pneumoniae* accession number MT078679. Analysis of bacterial extract S20 through GC-MS indicated the presence of 8 compounds of diverse nature and structure. Present study suggests that wastes of pharmaceutical and poultry feed industry may have antibiotic producing bacteria. These bacteria could be utilized for the production of antibiotics. *B. cereus* and *K. pneumoniae* isolated from wastes of poultry feed and pharmaceutical industries have the potential to produce antibiotics and could be used to control the microbial growth.

Keywords: antibiotic producing microorganisms, antibacterial activity, zone of inhibition, soil sample, well diffusion method, spread plate technique.

Resumo

Muitos microrganismos do solo, ou seja, bactérias e fungos produzem metabólitos secundários chamados antibióticos. Eles são usados para tratamento de algumas doenças bacterianas, fúngicas e protozoárias em humanos. Há necessidade de isolamento de um amplo espectro de antibióticos de microrganismos devido ao surgimento de resistência aos antibióticos. No presente estudo, duas bactérias produtoras de antibióticos, *Klebsiella pneumoniae* e *Bacillus cereus*, foram isoladas da indústria farmacêutica e de ração avícola de Hattar, Haripur, Paquistão. Um total de 10 amostras de resíduos foi coletado de diferentes indústrias (mármore, ghee, sabão, mineral, aço, ração para aves, farmacêutica, Qarshi, cosmética e vidro). Trinta e três cepas bacterianas foram isoladas de resíduos industriais dessas dez diferentes indústrias. Quatorze das 33 cepas bacterianas exibiram atividades antimicrobianas contra

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pelo menos um dos micróbios de teste considerados neste estudo, incluindo *Escherchia coli*, *Staphylococcus aureus* e *Salmonella typhi*. As bactérias foram isoladas pela técnica de placa de diluição em série padrão. A caracterização morfológica dos isolados foi feita por coloração de gram. Nove isolados bacterianos de 14 foram inicialmente identificados como *B. cereus* e cinco como *K. pneumoniae* por meio de caracterização bioquímica. As atividades antibacterianas foram testadas pelo método de difusão em poço. O número máximo de bactérias produtoras de antibióticos foi isolado da indústria farmacêutica e de ração avícola com base nos resultados da triagem primária, os isolados mais potenciais S9, S19, S20, S22 e S23 foram selecionados para a triagem secundária. A atividade máxima contra *E. coli* e *S. aureus* foi registrada pelo isolado bacteriano S19, ou seja, zonas de inibição de 6,5 mm e 9 mm, enquanto S20 mostrou zonas de 7,5 mm e 6 mm, respectivamente. A identificação molecular foi realizada com base na análise da sequência 16S rRNA. Finalmente, os isolados foram identificados como *B. cereus* número de acesso LC538271 e *K. pneumoniae* número de acesso MT078679. A análise do extrato bacteriano S20 por meio de GC-MS indicou a presença de oito compostos de natureza e estrutura diversas. O presente estudo sugere que resíduos da indústria farmacêutica e de ração para aves podem conter bactérias produtoras de antibióticos. Essas bactérias podem ser utilizadas para a produção de antibióticos *B. cereus* e *K. pneumoniae* isolados de resíduos de rações de aves e indústrias farmacêuticas têm potencial para produzir antibióticos e podem ser usados para controlar o crescimento microbiano.

Palavras-chave: microrganismos produtores de antibióticos, atividade antibacteriana, zona de inibição, amostra de solo, método de difusão em poços, técnica de espalhamento em placa.

1. Introduction

Microorganisms produce several secondary metabolites which play a very important role in human health. These bioactive compounds are present as bacteriosins and mycotoxins produced by bacteria and fungi. Actinomycetes have also been used for the production of important drugs like erythromycin, vancomycin, streptomycin and actinomycin etc (Falkinham 3RD et al., 2009). Most of the antibiotics are produced by soil microorganisms. Natural soil provides a best medium for the growth of antibiotics producing microorganisms. (Demain and Fang, 2000, Abbas et al., 2014). These Antibiotics have been incredibly useful for the treatment of some of the bacterial, fungal and protozoal diseases of human. The demand for new antibiotics has been increasing day by day due to the emergence of drug resistant pathogens. Pharmaceutical industry all over the world is producing more than one million tons antibiotics per annum. About five thousand and five hundred antibiotics have been discovered due to constant efforts made in this field (Kaur et al., 2014). Presently several micro-organisms are grown in artificial medias for the search of antibiotic producing microorganisms. A Streptomyces strain SJE177 was isolated from soil which exhibited the production of actinomycin (Euanorasetr et al., 2010). Waste soil is also considered a source of microorganisms and bioactive compounds producing micro-organisms were isolated from waste soils of India (Singh and Mishra, 2013). A large amount of microorganisms are found in the soil, as it is the most suitable environment for their survival (Chaudhary et al., 2013). Two most known inhibitory bacteria are *Bacillus* and *Actinomycetes* which are found predominantly in the soil which show antibiotic production by producing Bacitracin and Actinomycin correspondingly.

B. pumilus and *B. subtilis* have been reported to show antimicrobial activity against *S. aureus* and *Micrococcus luteus* (Tabbene et al., 2009). For better growth of microorganisms, the most favourable conditions of pH, temperature, organic matter and moisture are required. Aminoglycosides, Penicillin, Macrolides, Glycopeptides, Cephalosporins and Tetracyclins are most common antibiotics (Demain, 2009). *Bacillus* species are found predominant in soil which inhibit the growth of a variety of microorganisms due to the formation of endospores which are resistant and also have importance for the production of essential antibiotics such as bacitracin etc. Different studies confirmed *Bacillus* species to produce antimicrobial compounds having pharmaceutical and biotechnological importance (Awais et al., 2007). Actinomycetes from soil are considered the best source of the production of a number of drugs including from actinomycin and streptomycin, to vancomycin and erythromycin. The variety of terrestrial actinomycetes are important in several areas of medicine and science for the production of antibiotics. Majority of the biologically active compounds isolated from actinomycete, have been arranged into numerous classes which are based on their structures including b-lactam i.e cephalosporins, ansamycins (e.g., rifampin). Erythromycin are macrolides, tetracycline while kanamycin and streptomycin are amino glycosides (Kayode and Sani, 2008). *Streptomyces griseus* is an additional microorganism found in the soil, which has the ability to produce bioactive compound known as streptomycin that shows the bactericidal activity against various harmful bacteria such as *M. tuberculosis* (Thakur et al., 2007). Over the ancient decades the bioactive compounds of these microorganisms have been utilized against number of bacterial infections. But as pathogens showed resistance to those previously available antibiotics, multi drug resistance is the most important problem of these days that is faced by everyone. So, there is a great requirement to discover novel antibiotics or to find out some other alternatives to fight against these pathogenic bacteria (Nithya and Pandian, 2010).

The objective of this study was to isolate antibiotic producing microorganism from different industrial wastes of Hattar, Haripur. Another objective of study was to find the predominantly found microbes in the waste soil samples of ten different industries which could how antibacterial activity against *S. aureus*, *E. coli* and *S. typhi*.

2. Material and Methods

2.1. Sample collection, preparation and isolation of bacteria

For the isolation of antibiotic producing microorganisms, industrial waste soil samples were collected from ten different industries of Hattar. Approximately ½ kg of waste soil samples were collected for further processing. Samples were collected from the soil of crust and depth of 4 inches with the help of sterile spatula and placed in sterile polythene bags for transportation to Microbiology laboratory. Sample preparation was done through serial dilutions in a laminar air flow. Five sterile test tubes were taken for each sample, marked and labelled for each sample. A soil suspension is made by adding 1g of dried soil sample in 10ml of distilled water in a first test tube and is vortexed. This stock solution was then diluted serially up to the dilution of 10^{-5} and 0.1mL of diluted sample was inoculated on the sterile nutrient agar plates then incubated at 37ºC for 24 hours in an incubator. For the isolation of pure cultures of bacterial strains, the sub culturing was done by using the sterile loop to pick the bacterial colonies having clear boundary and were streaked on the fresh nutrient agar plates by using streak plate method and were incubated at 37ºC for 24 hours. The purified colonies were preserved using standard preservation methods.

2.2. Microscopic and Biochemical characterization of selected bacteria

After isolation of bacterial strains on Nutrient agar plates, strains were identified and characterized by morphological, cultural and biochemical tests using Bergay's manual as a reference (Vos et al., 2009). Microscopic and biochemical tests were performed to recognize the isolated strains. For microscopic identification gram staining technique was performed. Biochemical tests were used for the identification of bacterial isolates as described by Bergey's manual *i.e.* Catalase, Citrate, Oxidase, Urease, Indole etc.

2.3. Antibacterial activity testing

Antibacterial activity of the isolated bacterial strains was checked against three test organisms. Two Gram negative and one gram positive bacteria were used as test pathogens in this study and were collected from NESCOM hospital, Islamabad. For testing of antibacterial activity three test pathogens including *E. coli, S. aureus* and *S. typhi* were grown in nutrient broth. After inoculating LB with the test organisms and isolated bacterial strains in separate test tube, these were incubated at 37ºC for 24 hours. Then after 24 hrs of incubation of the isolated bacterial strains and test organisms, antibacterial activity was checked through well diffusion method by using Mueller Hinton Agar. Well diffusion method was performed to observe the antibacterial action of the isolated strains. In this method sterile cotton buds were used to swab all three test pathogens on 3 different MHA plates, then by using sterile cork borer, four wells of the size of 9 to 10 mm, were made on each plate. About 100-150 mL of culture supernatant of different isolated strains were loaded into different wells of each plate and incubated at 37ºC for 24 hours. The zone of inhibition was checked after 24 hours of incubation. Finally, the zones was measured with the scale.

2.4. Molecular identification of bacteria by 16S rRNA amplification and sequencing

Bacterial DNA was extracted according to the TIANamp Bacteria kit protocol by following the manufacturer's protocol. The universal primers (Forward primer): 5' CCTACGGGAGGCAGCAG 3') as well as reverse primer (5' TGGACTACCAGGGTATC 3') were used for the amplification of the 16S rRNA gene fragment. The PCR programme was as follows: Initial denaturation, 94°C for 5 min followed by 30 cycle in which one cycle consist of three different temperature 94°C for 30 second, 55°C for 30 second and 72°C for 1min and final cycle for 72°C for 10 min. The rRNA reaction mixture contained 1 µl of DNA template (10 ng), 0.4 microliter of each universal forward and reverse primers, 4 microliter of 6X Master Mix (containing Taq-DNA polymerase, dNTPs, $MgCl_2$ along with reaction buffers) and 14.2 µl of autoclaved distilled water. The PCR product was observed by using gel electrophoresis.

2.5. Sequencing and analysis of 16S rRNA

The amplified 16S rRNA gene fragment was purified and sequenced using DNA sequencing services (Macrogen, Korea). 16S rRNA sequence was analyzed by using Basic local alignment search tool (BLAST) available from the website of National Center for Biotechnology information (https://blast.ncbi.nlm.nih.gov) to identify the identical matches with existing characterized reference sequences.

2.6. Phylogenetic analysis of isolated bacterial strains

The sequences of the close hits were retrieved from NCBI and performed multiple sequence alignment by using Clustawl W. The aligned files were then exported in mega format. Neighbor-joining tree was constructed in MEGAX using default parameters and bootstrap values of 1000.

2.7. Gas Chromatography-Mass Spectrometry Analysis of Bacterial isolate S20

The crude extracts of samples were used to evaluate their chemical composition using GC-MS. A 20-min run was conducted from initial temperature of 40 °C to the final temperature of 250 °C. The spectrum was noted in the range of 40-600m/z. Peaks of various compounds eluted from the column of GC were recorded along with their retention time. Data was correlated with mass spectra of these compounds and database was searched for similar compounds with same retention time and molecular mass. Bioactivities of already reported natural compounds were studied and a comparison was made to correlate the activities of bacterial extract with their constituents.

2.8.Statistical analysis of zones of inhibition

One-way ANOVA was applied on zones of inhibition data for antibacterial activities by using SPSS software. Tukey test was used as a Post hoc test to compare each group with all the others.

3. Results

3.1. Isolation and identification of bacterial isolates having antibacterial activity against test strains

Thirty-three bacterial strains were isolated from industrial wastes of ten different industries (Marble, Ghee, Soap, Mineral, Steel, Feed, Pharmaceutical, Qarshi, Cosmetic and Glass) (Figure . 1). Fourteen out of total thirty-three bacterial strains showed antimicrobial activities against at least one of the test microbes considered in this study including *E. coli*, *S. aureus* and *S. typhi* (Figure 2, Table 1). Gram staining and different biochemical tests exhibited the presence of nine isolated antibacterial strains as *Bacillus sp.,* while the biochemical tests of five samples revealed identity with *klebsiella sp.* (Table 2). *klebsiella sp.* isolated from pharmaceutical industry was gram negative as it showed pink rods on gram staining and positive for catalase, citrate, urease, fermentation tests (Figure 3, Table 2). The oxidase indole and motility tests exhibited by *klebsiella sp.* were negative (Table 2). *Bacillus* strains isolated from poultry feed industry were gram positive rods (purple rods) and positive for catalase, citrate and motility, while negative for oxidase, urease and indole tests (Figure 3, Table 2). Fermentation tests exhibited by *Bacillus* strains were positive for sucrose, fructose and glucose, while negative test with mannitol (Table 2). The molecular identification was carried out on the basis of 16S rRNA sequence analysis. The results

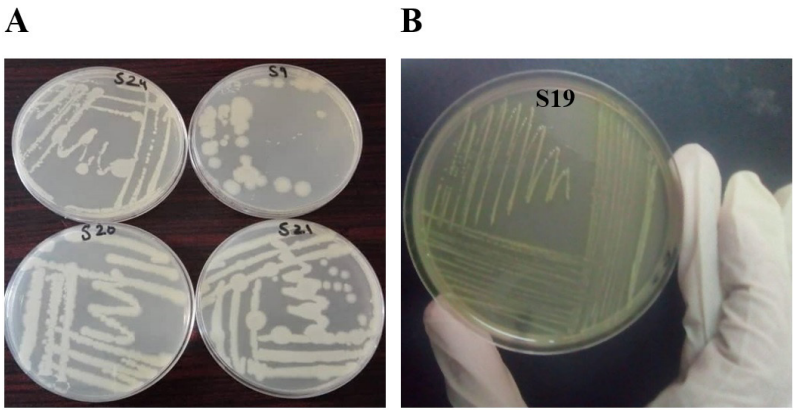

Figure 1. Growth of bacterial strains on nutrient agar. (A) Strain S9 isolated from waste of soap industry, S21 from feed industry, S20 and S24 from Pharmaceutical industry; (B) S19 strain isolated from poultry feed waste.

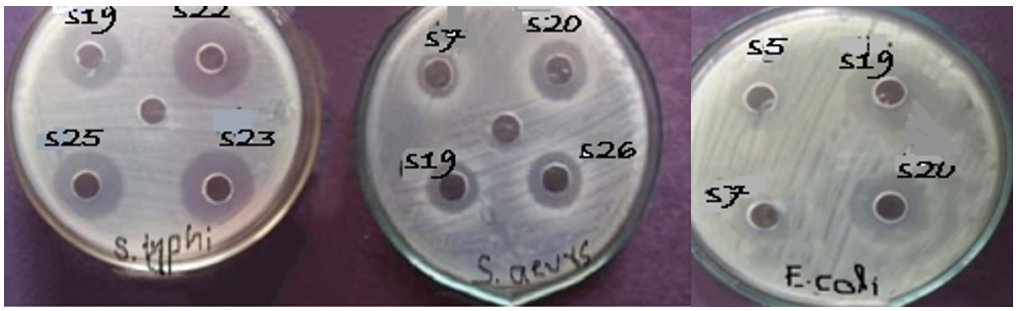

Figure 2. Antibacterial activity of isolated strains as depicted by clear zones of bacterial isolates. S5, S7, S19, S20, S22, S23, S25, S26 showed the isolated bacterial strains from industrial waste samples for testing antibacterial activities against *S. typhi, S.aureus* and *E.coli.*

Table 1. Zones of clearance exhibited by different bacterial isolates against test pathogens (*E. coli, S. aureus* and *S. typhi*).

S.No	Zones of clearance exhibited by different bacterial isolates against three pathogens (mm)												
	Test pathogens	**S2**	**S5**	**S7**	**S15**	**S19**	**S20**	**S21**	**S22**	**S23**	**S25**	**S26**	**S27**	**S33**
1	*E. coli*	**5.5**	2	1	**0**	6.5	**7.5**	0	6	**5**	0	0	**0**	2
2	*S. aureus*	**0**	5.5	6	**4**	9	**6**	4	4.5	**4**	5.5	6.5	**5.5**	4.5
3	*S. typhi*	**0**	1	2.5	**0**	5.5	**0**	0	7.5	**7.5**	7.5	5.5	**3.5**	2

Note: S2, S5, S7, S15, S19, S20, S21, S22, S23. S25, S26, S27 and S33 are different bacterial isolates. *S. aureus* is *Staphylococcus aureus*. *S. typhi* is *Salmonella typhi*. *E. coli is Escherchia coli.* Text highlighted in bold exhibited the zone of inhibition by *K. pneumonia* against three test pathogens *E. coli, S. aureus* and *S. typhi.* while normal text showed the zones of inhibition by *B. cereus* against three test pathogens.

Table 2. Biochemical, Motility and Morphological tests of different isolated bacterial strains.

S.No	Biochemical tests	S2	S5	S7	S15	S19	S20	S21	S22	S23	S25	S26	S27	S33
1	Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+
3	Citrate	+	+	+	+	+	+	+	+	+	+	+	+	+
4	Urease	+	-	-	+	-	+	-	-	+	-	-	+	-
5	Indole	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Suc. Ferm.	+	+	+	+	+	+	+	+	+	+	+	+	+
7	Fruc. Ferm.	+	+	+	+	+	+	+	+	+	+	+	+	+
8	Gluc. Ferm.	+	+	+	+	+	+	+	+	+	+	+	+	+
9	Mannitol	+	-	-	+	-	+	-	-	+	-	-	+	-
10	Motility test	NM	M	M	NM	M	NM	M	M	NM	M	M	NM	M
11	Morphology Gram staining	GN	GP	GP	GN	GP	GN	GP	GP	GN	GP	GP	GN	GP

Note: S2, S5, S7, S15, S19, S20, S21, S22, S23. S25, S26, S27 and S33 are different bacterial isolates. NM is employed for Non motile, M for Motile. + sign shows positive test, - sign shows negative test result. Suc. Ferm is employed for Sucrose fermentation, Fruc. Ferm. is employed for fructose fermentation. Gluc. Ferm. is employed for Glucose fermentation. Different bacterial isolates have been isolated from industrial wastes of ten different industries.

Table 3. Identification of antibacterial activity showing bacteria.

S.no	Source	Isolates	16SrRNA amplified region length	% Identity/ similarity	NCBI Accession no.
1	Poultry feed wastes	S19	490 bp	99% with *Bacillus cereus*	LC538271
2	Pharmaceutical industrial wastes	S20	271 bp	98% with *Klebsiella pneomoniae*	MT078679

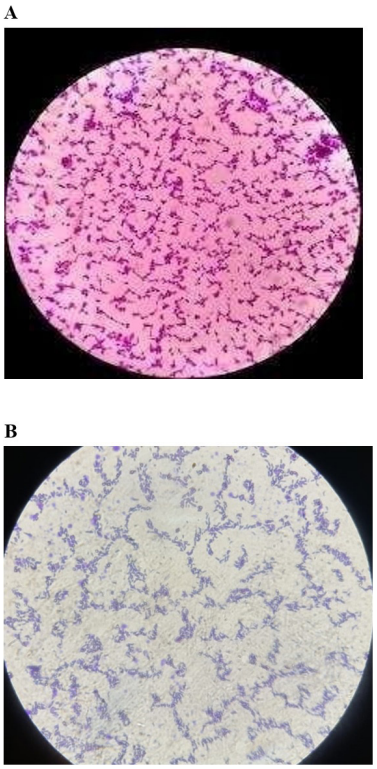

Figure 3. Colony morphology of isolated antibiotic producing bacterial strains. Figure (A) shows gram negative rods while spore forming purple rods have been exhibited in figures (B).

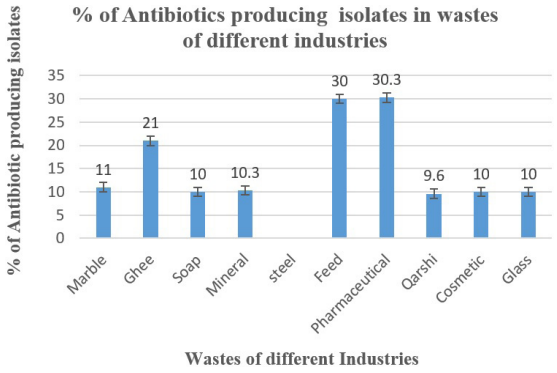

Figure 4. Percentages of antibiotic producing isolates in the wastes of different industries of Hattar, Haripur. Error bars indicate standard errors of mean values (n=3).

of DNA sequencing were submitted to GenBank with the accession number of MT078679 for *klebsiella pneumonia* (S20) and LC538271 (S19) for *Bacillus cereus* (Table 3). Finally the isolates were identified as *Bacillus cereus* accession number LC538271 and *klebsiella pneumonia* accession number MT078679.

The highest number of antibiotic producing bacteria were isolated from the Pharmaceutical and feed industry which is depicted in Figure 4 while no bacterium was isolated from the wastes of steel industry. Although some bacteria were also isolated from the wastes of other industries including ghee, marble, cosmetics, soap, mineral, Qarshi, cosmetics

and glass but their number was lower as compared to the poultry feed and pharmaceutical industries (Figure 4). The presence of antibiotic producing bacteria depends on the presence of some energy source or nutrients. In the waste samples of steel industry, no nutrients or energy source is available for the growth of bacteria, hence no bacteria were isolated from wastes of steel industry. Less number of isolated bacteria in wastes of marble, cosmetics, soap, mineral, Qarshi, cosmetics and glass industries also show low nutrients and less amount of energy sources present there. Like all living things, bacteria need food, water and the proper environment to live and grow. A bacterium must have an energy source, a source of carbon and other required nutrients, and a permissive range of physical conditions such as O_2 concentration, temperature, and pH.

The experiment for testing antibacterial activities was repeated thrice. Significant values of zones of inhibition were observed at 95% confidence interval (mean difference was significant at 0.05% level). The bacterial strains isolated from Poultry feed and Pharmaceutical industries exhibited significantly higher values of inhibition zones as compared to the Marble, Ghee, Soap, Mineral, steel, Qarshi, cosmetics and glass industries for at least one pathogen. It was depicted by sig.0.00 values ($0.00 \leq 0.05$) obtained by performing ANOVA with Tukey test by using SPSS. No bacterial strain was isolated from waste samples of steel industry.

3.2. GC-MS analysis of bacterial extracts

GC-MS analysis of ethyl alcohol extracts of Bacterial sample S20 exhibited the presence of bioactive compounds. The presence of several compounds with known biological activity was found in the bacterial extracts analyzed and references are provided in Table 4 describing where compounds observed in such type of study were previously found.

4. Discussions

Soil is known to harbor antibiotic producing microorganisms which may be of great medicinal importance that include *M. s roseus, Brevibacterium sp., B. subtilus, B. anthracis, B. cereus* etc. (Rafiq et al., 2018). In order to explore the potential of different industrial wastes of Hattar, Haripur to produce antibiotic producing bacterial strains, we carried out a study to identify any bacteria with potential to synthesize bioactive compounds against 3 pathogenic bacteria (*S. aureus, S. typhi and E. coli*). Results revealed the presence of two antibacterial activity exhibiting bacterial strains *K. pneomonae and B. cereus* from the wastes of pharmaceutical and poultry feed industry (Table 3). The isolated bacteria belonged to Phylum Proteobacteria and firmicutes. Several researchers have

Table 4. Major constituents of bacterial extracts S20, as depicted by GC-MS analysis.

S. No	Retention time	Compound name	Mol. Weight	Formula	Biological Activity
1	3.08	3 Chloropropionic acid 2 Pentadecyl ester	318g/mol	C18H3502Cl	Antibacterial activity of its derivative is reported (Fadhil et al., 2018)
2	3.08	Octadecane 1 Chloro	288g/mol	C13H37Cl	Antibacterial activity of its derivative is reported (Boussaada et al., 2008)
3	3.44	Benzyl Isoproponyl Ether	148g/mol	C10H120	Antibacterial and antifungal activity of its derivative is reported (Mohammed et al., 2016)
4	3.44	Benzene (3, 3 Dimethylbutyl	162g/mol	C12H18	Antimicrobial activity of its derivative is reported (Wintola and Afolayan, 2017)
5	3.55	Propionic acid 2 (Amnooxy)	105g/mole	C3H703N	Antimicrobial activity of its derivative is reported (Katariya et al., 2019)
6	3.55	2 Propanone 2- (2,4 Dimethylphenyl hydrazone)	176g/mole	C11H16N2	Antimicrobial activity of its derivative is reported (Hassan et al., 2017)
7	3.64	Benzene Eicosyle	358g/mole	C24H46	Antimicrobial activity of its derivative is reported (Wintola and Afolayan, 2017)
8	3.64	8 Phenyl Octanoic acid	220g/mole	$C14H20O_2$	Antibacterial and antifungal activity of its derivative is reported (Mohammed et al., 2016)

The compounds showed resemblance with the natural products of bacterial origin (Faridha Begum et al., 2016).

indicated the importance of Proteobacteria, Firmicutes as antibiotic producers. *Bacillus* species and some other spore forming bacteria carry genes for the production of antibiotics (Stein, 2005). Bacitracin produced by *Bacillus* species inhibited both *E. coli and S. aureus*. A great diversity of secondary metabolites are produced by *Bacillus* species isolated from soil (Awais et al., 2007). Hassan et al., (2014) also conducted similar study for the isolation of antibiotic producing microorganisms against different test pathogens.

In the present study antibiotic producing bacteria were isolated from waste soil of different industries of Hattar, Haripur. *Bacillus cereus* isolated from poultry feed industry exhibited clear zone of inhibition against test pathogenic microorganisms (*E. coli, S.aureus and S. typhi)*. This is demonstrated in Table 1 by observing the zones of inhibition of isolated bacterial samples S19 and S22 against test pathogens. This finding is in accordance with Ahmed et al. (2013), who screened soil microorganisms for detection of antibiotic producing bacteria. They identified *Bacillus* species as antibiotic producing bacteria from soil (Ahmed et al., 2013). Screening of microorganisms has been done through relatively rapid and simple methods for antibiotic production. Soil bacteria and fungi may show an important feature of antibiotic production. The bacteria isolated from soil show antibiotic activity under normal growth conditions and are found to inhibit some gram-positive as well as gram-negative organisms. A neighbor joining tree depicting the phylogenetic relationship of isolated bacterial strains S19 and S20 revealed the relationship of isolated bacteria with different strains of *B. cereus* and *K. pneumonae* (Figures 5 & 6).

In the present study we also identified *K. pneumonia*. Bacterial samples S20 and S23 were identified as potential antibiotic producing strains of *k. pneumonia*. Zones of inhibition of 7.5mm and 4mm were exhibited by *K. pneumonia* (Sample S20 isolated from wastes of pharmaceutical industry) against test pathogens *E. coli and S. aureus* (Table 1). Another isolated strain of *K. pneumonia* S23 also exhibited zones of inhibition against test pathogens *E. coli, S. aureus and S. typhi*. These isolated bacterial strains of *K. pneumoniae* and *B. cereus* could be utilized for the production of antimicrobial compounds against *S. aureus*, *E. coli* as well as *S. typhi.*

Industrial wastes of pharmaceutical and feed industry may also show other antibiotic producing microorganisms. Present study is in accordance with Kishore and Vijayalakshmi (2018). They isolated micro-organisms from sewage samples collected from Andhra Pradesh, Visakhapatnam. Three strains of antibiotic producing bacteria were isolated (AntC1, AntC3 and AntC8), which exhibited antibacterial activity against *S. aureus* as well

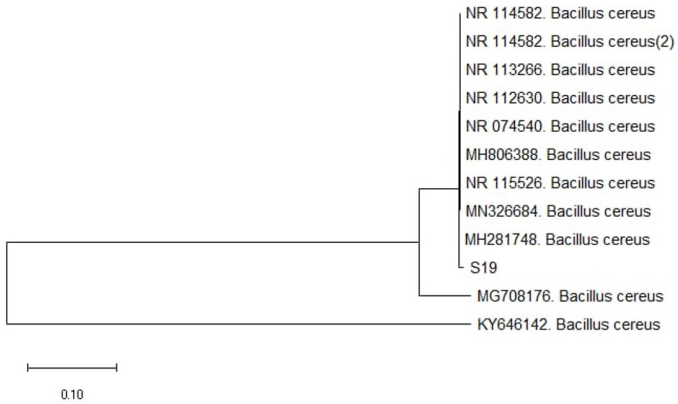

Figure 5. A neighbor-joining tree depicting the phylogenetic relationship of isolated bacterial strain S19. The tree has been constructed using MEGA X.

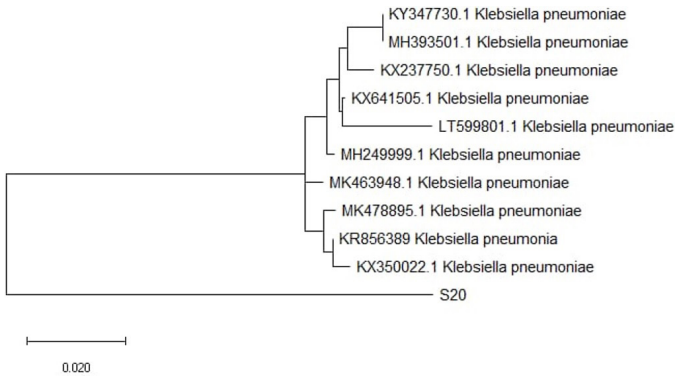

Figure 6. A neighbor-joining tree depicting the phylogenetic relationship of isolated bacterial strain S20. The tree has been constructed using MEGA X.

as *E. coli* (Kishore and Vijayalakshmi, 2018). A similar study was also conducted by Kumar et al. (2018), who screened different bacteria to observe the antagonistic and antibacterial activity against several test organisms through various methods. Four isolated strains of bacteria were found to show high antibiotic activity against test organisms (*B. subtilis* and *S. aureus)* (Kumar et al., 2018). Gislin et al. (2018) revealed a study on antibiotic producing micro-organisms. They isolated bacteria from soil and tested their antibiotic ability against six human pathogens including *S. aureus, E. coli, P.aeruginosa, Enterococcus sp, K. pneumoniae,* and *Acineto-bacter sp*. Each isolate exhibited antibacterial activity against *S. aureus and species of Enterococcus* (Gislin et al., 2018). Abbas et al. (2014) revealed a study on soil samples collected from different locations of Bangalore for the isolation and characterization of novel antibiotic producing microorganisms against *Bacillus* strains. They identified *Bordetella*, *Achromobacter* and *Streptomyces* as antibiotics producers (Demain and Fang, 2000). Rafiq et al. (2018) also demonstrated the presence of antibiotic producing bacteria and fungi from soil (Hassan et al., 2017). The isolated strains of bacteria were identified as *B. subtilis, M. roseus, B. anthracis Brevibacterium* sp., and *B. cereus* through biochemical characterization and fungal isolates were recognized as, *Cladosporium cladosporides, Epicoccum nipponicum, Tricho-cladium opacum A. niger* and *Rhizocotania* sp.

To identify the bioactive compounds of the isolated bacterial extract S20, the GC-MS analysis was performed which indicated the presence of esters, alcohols, alkanes, amines and other derivatives in the isolated bacterial extract (Table 4). These findings are in accordance with previous reports. Production of bioactive compounds in bacterial strains isolated from wastes of Pharmaceutical and poultry feed industry could be used for the preparation of antibiotics against some resistant pathogens. Present research project also demonstrates the presence of potential antibiotic producing microorganisms in the waste of pharmaceutical and feed industry. It may be inferred that more research may be conducted by increasing the sample size and selecting different areas to isolate some other antimicrobial bacterial strains. This may broaden the horizon of research for antibiotic producing microorganisms.

5. Conclusions

Wastes of pharmaceutical and poultry feed have the potential to produce bacteria exhibiting activities against pathogens such as *E. coli*, *S. aureus* and *S. typhi*. The isolated strain (S19) of *B. cereus* accession number LC538271 could be utilized for the production of bioactive compounds against *S. aureus, Salmonella spp. and S. typhi*. Similarly identified strain of *K. pneumoniae* accession number MT078679 could be used to produce antibacterial compounds against *S. aureus and E. coli*. Understanding the mechanism of action of isolated bioactive compounds from these bacteria will broaden the horizon of research in antibiotic drug discovery. Future work will be concentrated on the isolation and detailed characterization of bioactive compounds from different bacterial strains.

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