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Bioactivity of *Alcaligenes* spp. isolated from cow dung against certain human pathogens

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Abstract

Seven bacterial strains were isolated from cow dung, morphological and biochemical investigations identified, KD109 and KD110 as *Alcaligenes fecalis and Alcaligenes Latus*. These two isolates were screened for their antagonistic activity against 14 test organisms using cross-streak method. The preliminary screening revealed that isolate KD109 inhibit *Vibrio cholerae* (MTCC 3904), *Salmonella typhi* (MTCC 3216), *Escherichia coli* (SGPGI), *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis* (MTCC 441) and *Bacillus cereus* (MTCC 6728), while isolate KD110 inhibit *Salmonella typhi* (MTCC 3216), *Escherichia coli* and *Bacillus cereus* (MTCC 6728). This study indicates that these *Alcaligenes* spp. may be up-hold to industrial level for production of antimicrobial agent, which should be further analyzed for its possibility to be used as therapeutic agent.

Key words: Alcaligenes* spp., *Cow dung, Cross-streak method, Antimicrobial activity, Human Pathogens

Introduction

Secondary metabolites are produced by the microorganisms to ensure their survival in a competing environment. These naturally produced chemical compounds have played a key role in the development of antimicrobial drugs Kleinkauf and Dohren (1990); Esikova *et al.,* (2002); Ilic *et al.,* (2007); Gupta and Rana (2016a), with many drugs present in the market now days are of microbial origin Ilic *et al.,* (2007); Harvey (2008); Butler *et al.,* (2014); Gupta and Rana (2016b). Different habitat have been explored for instance, *Lactobacillus* strains isolated from camel milk showed antibacterial activity against *Bacillus cereus,* Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli, Salmonella typhimurium* Abbas and Mahasneh (2014), *Bacillus lentus, Micrococcus roseus, Enterobacter aerogene, Bacillus pumillus, Bacillus alvei* were isolated from the soil showed bioactivity against *Shigella* spp., *Staphylococcus aureus, Pseudomonas* spp., *Proteus* spp. Abdulkadir and Waliyu (2012),marine microorganisms such as *Brevibacterium* sp., *Moraxella* sp. were also find effective against *Bacillus subtilis* and *Staphylococcus aureus* Al-Zereini (2014), *Enterococcus faecium* isolated from fermented Spanish sausage was found effective against *Listeria innocua* BL86/26 Aymerich *et al.,* (1996), Sponge associated symbiotic bacterias were also explored, *Micrococcus luteus,* a symbiont to *Xestospongia testudinaria* was found effective against *Bacillus subtilis, Escherichia coli* and *Klebsiella pneumonia* Gupta *et al.,* (2016). But in spite of that, even at present, pace of approved antimicrobial agents against the rate of resistance is lagging, hence emergence of drug resistant bacteria is an alarming situation worldwide Pelaez (2006); Fischbach and Walsh (2009); Bhatta and Kapadnis (2010); Dandekar and Dandekar (2010); Eggleston *et al.,* (2010); Donadio *et al.,* (2010); Davies and Davies (2010); Ascencio *et al.,* (2014); Kapley *et al.,* (2016). Researchers around the world are continuing their effort to isolate bacteria capable of producing bioactive compounds from a range of different habitats like soil, marine environment and even from the gut of animals and insects Gupta and Rana (2016a). Cow dung, is the waste generated after the digestion of consumed food material, posses wide variety of microorganisms Nene (1999); Morgavi *et al.,* (2010); Randhawa and Kullar (2011); Waziri and Suleiman (2013);

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Adeniyi *et al.,* (2015). Antifungal substances isolated from cow dung inhibits the growth of coprophilous fungi Dhama *et al.,* (2005); Joseph and Sankarganesh (2011); Dhama *et al.,* (2013). *Eupenicillium bovifimosum,* that produces patulodine-like compounds viz. CK2108A and CK2801B was also isolated from cow dung Dorothy and Frisvad (2002); Lehr *et al.,* (2005). *Enterococcus faecalis* V24, isolated form cow dung, produces a heat stable, largely hydrophobic antimicrobial substance possessing antimicrobial activity against pathogenic Gram-negative bacteria Laukova *et al.,* (1998). Teo and Teoh (2011) isolate a strain from cow dung i.e. K4 showing antibacterial activity against *E. coli*. Shrivastava *et al.,* (2014) evaluate water, ethanol and n-Hexane extract of whole cow dung against *Candida* sp., *E. coli*, *Pseudomonas* and *Staphylococcus aureus* revealing their antimicrobial properties.

Alcaligenes spp. has also been evaluated for their antimicrobial properties against *Staphylococcus aureus* FDA209P, *S. aureus* No. 5 (MRSA)**, *S. epidermidis* IID866, *S. epidermidis* No. 17 (MRSE)**, *Bacillus subtilis* ATCC6633, *Salmonella enteritidis* 1 891, *Escherichia coli* 0~1, *Pseudomonas aeruginosa* ATCC8689, *Klebsiella pneumoniae* ATCC 10031 Kamigiri *et al.,* (1996) but *Alcaligenes* spp. isolated from cow dung exhibiting bioactivity has not been reported. Hence, the present study was undertaken to explore cow dung as a source for the isolation of *Alcaligenes* spp. exhibiting antimicrobial properties.

Material and Methods

Sample collection: Dung sample of desi cow breed (Krishna) was collected from Saharanpur in the month of December and stock in sterile container, transported, to laboratory and stored at 4°C for further studies Gupta and Rana (2016a).

Isolation of Bacterial Species: Bacteria were isolated on nutrient agar medium (NAM) by standard serial dilution method. Stock solution was prepared by mixing 1g of cow dung sample with 9ml of normal saline. The stock solution was diluted up to 10^{-8}. 100 µl of solution from each dilution was spread on nutrient agar medium followed by incubation in upright position at 37°C for 24h Das (2010); Gupta *et al.,* (2016); Gupta and Rana (2016a); Gupta and Rana (2016b).

Morphological characterization of *Alcaligenes* spp: Morphological characterization of bacteria was done in accordance with standard Gram-staining technique Beveridge (2001); Gupta *et al.,* (2016).

Biochemical characterization of *Alcaligenes* spp.: All the isolates were subjected for carbohydrate fermentation test, gelatine liquefaction, starch hydrolysis, citrate utilisation, catalase production, indole and MR-VP for biochemical characterization as described by Holt *et al.,* (1994); Das (2011).

Bioactivity of *Alcaligenes* spp. by cross-streak method: Cross streak method Mohseni *et al.,* (2013) was used to evaluate the bioactivity of *Alcaligenes* spp. against 14 test organisms i.e., *Vibrio cholera* (MTCC 3904), *Salmonella typhi* (MTCC 3216), *Escherichia coli* (SGPGI), *Staphylococcus aureus* (MTCC 7443), *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 6728), *Proteus vulgaris* (MTCC 426), *Enterococcus faecalis* (MTCC 439), *Pseudomonas aeruginosa* (MTCC 424), *Escherichria coli* (MTCC 118), *Shigella flexneri* (MTCC 1457), *Salmonella typhimurium* (MTCC 3231), *Streptococcus pyogenes* (MTCC 442) and *Staphylococcus aureus* (MTCC 3160). *A. fecalis* and *A. latus* were streaked onto NAM plates as a single streak in the centre and incubated at 37°C for 24h. The test bacterial strains were streaked perpendicular to the isolates on the NAM plates and incubated further at 37°C for 24 hours. The microbial inhibitions were observed by determining the distance of the inhibition zone between bacterial strain and test organisms.

Results and Discussion

Isolation of bacterial species: In the present study, to obtain cow dung sample, the desi cow was selected from a cow shed in Saharanpur (India) located at latitude of (29°5'04 N) and longitude of (77° 33' 04 E). Bacteria were isolated from dung sample by serial dilution method and total of 7

isolates namely KD104, KD105, KD106, KD107, KD108, KD109, KD110 were obtained.

Morphological and Biochemical characterization of *Alcaligenes* spp.:Morphological characterization of seven isolates revealed that isolate KD109 & KD110 were Gram-negative rods while isolate KD104 and KD106 are Gram-positive cocci and KD105, KD107 and KD108 were Gram-positive rod. Hence both the isolates (KD109 & KD110) were subjected for biochemical characterisation for probable identification of *Alcaligenes* spp. Isolate KD109 was not able to utilise glucose, lactose and sucrose, while KD110 was found to be positive for glucose utilisation and gelatine hydrolysation. Therefore, both the isolates i.e., KD109 and KD110 were identified as *A. fecalis* and *A. Latus* respectively Holt *et al.,* (1994); Das (2011). Detailed result of morphological and biochemical test are summarised in Table 1.

Table 1. Morphological and biochemical analysis of the bacterial isolates.

Test/Isolates	KD109	KD110
Gram Reaction	-	-
Shape	Rod	Rod
Glucose Fermentation	-	+
Lactose Fermentation	-	+
Sucrose Fermentation	-	-
Manitol Fermentation	+	-
Gelatine Liquification	+	+
Starch Hydrolysis	+	-
Indole	-	-
Methyl-Red	+	+
Vogeus-Proskeur	-	-
Citrate Utilisation	-	-
Catalase Production	+	+

Bioactivity of *Alcaligenes* spp. by cross-streak method: Out of the seven isolated strain, both the strains i.e. *A. fecalis* and *A. latus* were subjected to antimicrobial activity by cross-streak method against a panel of 14 test organisms having medical importance. Both the isolated strains *A. fecalis* and *A. latus* showed antibacterial activity against most of the test organisms. An important observation was recorded when *A. fecalis* (KD109) inhibited around 50% of the test organisms. Maximum inhibition (10mm) by KD109 was recorded against *Salmonella typhi* while minimum (6 mm) was recorded against *Escherichia coli* (SGPGI) and *Bacillus subtilis* (MTCC 441). Antimicrobial activity of *A. latus* (KD110) was recorded against two gram-negative (*Salmonella typhi and Escherichia coli*) and one gram-positive (*Bacillus cereus*) bacteria (Table 2). *A. fecalis* showed maximum inhibition (10 mm) against *Salmonella typhi* which is the best described cause of typhoid fever (TF) and paratyphoid fever Hsiao *et al.,* (2016) and minimum inhibition (6 mm) against *Bacillus subtilis* and *Escherichia coli.Escherichia coli* is one of the major medically important organisms that cause urinary tract infection as well as food poisoning and diarrhoea Alteri *et al.,* (2009); Teo and Teoh (2011). Both isolates were active against Gram-positive and Gram-negative test organisms, thereby indicating the secretion of broad spectrum antimicrobial agents. The difference in the sensitivity of Gram positive and Gram negative bacteria against *Alcaligenes* spp. may be due to the morphological differences in their outer membrane Gebreyohannes *et al.,* (2013). KD109 showed better result than KD110 for the reason that it inhibited more number of test organism to a greater extent. Zahir *et al.,* (2013) isolate *Alcaligenes* spp. from tannery waste and reported antimicrobial activity of ethyl acetate extract against *Escherichia coli, Bacillus subtilis*

Table 2. Inhibitory activity spectra of *Alcaligenes* spp. against test bacteria.

Isolates/ Test organisms	*V. cholera*	*S. typhi*	*E. coli*	*S. aureus*	*B. subtilis*	*B. cereus*	*P. vulgaris*	*E. fecalis*	*P. aeruginosa*	*E. coli*	*S. fexneri*	*S. typhimurium*	*S. pyogenes*	*S. aureus*
KD109	7mm	10mm	6mm	8mm	6mm	7mm	-	-	-	-	-	-	-	-
KD110	-	5mm	5mm	-	-	5mm	-	-	-	-	-	-	-	-

and *Staphylococcus aureus* with inhibition zone of 18mm, 16mm and 16mm respectively which is greater in comparison to the present study. This difference in activity may be due to the reason that crude extract were used by Zahir *et al.,* (2013). Although antimicrobial activity of *Alcaligenes* spp. has been reported earlier against many organisms such as *Bacillus subtilis* Li *et al.,* (2007), *Staphylococcus aureus* Li *et al.,* (2008), *Pseudomonas aeruginosa, Mycobacterium avium*, *Mycobacterium tuberculosis* Basic *et al.,* (2001), *Mycobacterium smegmatis*, *Pseudomonas aeruginosa* Zahir *et al.* (2013), *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 10876, *Shigella flexneri* ATCC 9199, *Enterobacter* (MDR strain) and *Serratia* sp. GMX1 (MDR strain) Kapley *et al.,* (2016). However, *Alcaligenes* spp. showing inhibition against *Salmonella typhi* is not well reported in the literature. Other authors showed that this species also produced compounds that can be of industrial use in the production of D-aminocyclases, semi-synthetic antibiotics (penicillin, cephalosporin, B-1015), hormones (lutein), bioactive peptides with immunostimulatory activities (cyclo-(l-Pro-Gly)5) and chemical pesticides Isono *et al.,* (1993); Tripathi *et al.*, (2000); Liaw *et al.,* (2003); Gayen *et al.,* (2007); Wang *et al.,* (2011). Besides, strains of *Alcaligenes faecalis* type N.C.T.C. 8764 and A.T.C.C. 9220 were antagonistic against *Escherichia* sp. Puah *et al.,* (2016). Antibiotic resistance among clinical pathogens i.e. *Salmonella typhi* , *Vibrio cholerae, Staphylococcus aureus* , *Escherichia coli* and *Bacillus cereus* has been reported recently against not only 1st and 2nd generation antibiotic but also against 4th generation of antibiotics; Mare and Coetzee (1964); Alteri *et al.* (2009); Puah *et al.,* (2016); Uppal *et al.,* (2017). Taking into consideration of these facts, isolated *Alcaligenes* spp. might be effective against these disease causing bacteria. The data of present study substantiates these several studies which made evident that *Alcaligenes* spp. is a potent antimicrobial agent producer able to inhibit bacterial species which are known to give rise to several diseases. The obtained results showed that two isolated strains (*A. fecalis* and *A. Latus*) have the potential for producing antimicrobial substances which is active against certain bacteria of clinical importance

References

Abbas, M.M. and Mahasneh, A.M., 2014. Isolation of *Lactobacillus* strains with probiotic potential from camels milk. ***African Journal of Microbiology Research***, 8(15):1645-1655.

Abdulkadir, M. and Waliyu, S., 2012. Screening and isolation of the soil bacteria for ability to produce antibiotics. ***European Journal of Applied Sciences***, 4(5):211-215.

Adeniyi, B.A., Adetoye, A. and Ayeni, F.A., 2015. Antibacterial activities of lactic acid bacteria isolated from cow faeces against potential enteric pathogens. ***African Health Sciences***, 15(3):888-895.

Alteri, C.J., Smith, S.N. and Mobley, H.L., 2009. Fitness of *Escherichia coli* during urinary tract infection requires gluconeogenesis and the TCA cycle. ***PLoS Pathogens***, 5(5):1-13.

Al-Zereini, W.A., 2014. Bioactive crude extracts from four bacterial isolates of marine sediments from Red Sea, Gulf of Aqaba, Jordan. ***Jordan Journal of Biological Sciences***, 7(2):133-137.

Asencio, G., Lavin, P., Alegría, K., Domínguez, M., Bello, H., González-Rocha, G. and González-Aravena, M., 2014. Antibacterial activity of the Antarctic bacterium *Janthinobacterium* sp.: SMN 33.6 against multi-resistant Gram-negative bacteria. ***Electronic Journal of Biotechnology***, 17(1):1-5.

Aymerich, T., Holo, H., Håvarstein, L.S., Hugas, M., Garriga, M. and Nes, I.F., 1996. Biochemical and genetic characterization of enterocin A from *Enterococcus faecium*, a new antilisterial bacteriocin in the pediocin family of bacteriocins. ***Applied and Environmental Microbiology***, 62(5):1676-1682.

Bacic, M.K. and Yoch, D.C., University of South Carolina, 2001. *Antibiotic composition from Alcaligenes species and method for making and using the same*. U.S. Patent 6,224,863.

Beveridge, T.J., 2001. Use of the Gram stain in microbiology. ***Biotechnic & Histochemistry***, 76(3):111-118.

Bhatta, D.R. and Kapadnis, B.P., 2010. Production optimization and characterization of bioactive compound against *Salmonella* from *Bacillus subtilis* KBB isolated from Nepal. ***Scientific World***, 8(8):19-29.

Butler, M.S., Robertson, A.A. and Cooper, M.A., 2014. Natural product and natural product derived drugs in clinical trials. ***Natural Product Reports***, 31(11):1612-1661.

Dandekar, T. and Dandekar, G., 2010. Pharmacogenomic strategies against microbial resistance: from bright to bleak to innovative. ***Pharmacogenomics***, 11(9):1193-1196.

Das, M., 2011. Chitinase produced by *Serratia marcescens* SMG isolated from decomposed *Volvariella volvacea*. ***African Journal of Microbiology Research***, 5(20):3220-3222.

Das, S., Ward, L.R. and Burke, C., 2010. Screening of marine *Streptomyces* spp. for potential use as probiotics in aquaculture. ***Aquaculture***, 305(1-4):32-41.

Davies, J. and Davies, D., 2010. Origins and evolution of antibiotic resistance. ***Microbiology and Molecular Biology Reviews***, 74(3):417-433.

Dhama, K., Chakraborty, S., and Tiwari, R., 2013. Panchgavya therapy (Cowpathy) in safeguarding health of animals and humans-a review. ***Research Opinions in Animal and Veterinary Sciences***, 3(6):170-178.

Dhama, K., Rathore, R., Chauhan, R.S. and Tomar, S., 2005. Panchgavya (Cowpathy): an overview. ***International Journal of Cow Science***, 1(1):1-15.

Donadio, S., Maffioli, S., Monciardini, P., Sosio, M. and Jabes, D., 2010. Antibiotic discovery in the twenty-first century: current trends and future perspectives. ***The Journal of Antibiotics***, 63(8):423-430.

Dorothy, E.T., and Frisvad, J.C., 2002. *Eupenicillium bovifimosum*, a new species from dried cow manure in Wyoming. ***Mycologia,*** 94(2):240-246.

Eggleston, K., Zhang, R. and Zeckhauser, R.J., 2010. The global challenge of antimicrobial resistance: insights from economic analysis. ***International Journal of Environmental Research and Public Health***, 7(8):3141-3149.

Esikova, T.Z., Temirov, Y.V., Sokolov, S.L. and Alakhov, Y.B., 2002. Secondary antimicrobial metabolites produced by thermophilic *Bacillus* spp. strains VK2 and VK21. ***Applied Biochemistry and Microbiology***, 38(3):226-231.

Fischbach, M.A. and Walsh, C.T., 2009. Antibiotics for emerging pathogens. ***Science,*** 325(5944):1089-1093.

Gayen, J.R., Majee, S.B., Das, S., and Samanta, T.B., 2007. Antibacterial and toxicological evaluation of ß-lactams synthesized by immobilized ß-lactamase-free penicillin amidase produced by *Alcaligenes* sp. ***Indian Journal of Experimental Biology,*** 45(12):1068-1072.

Gebreyohannes, G., Moges, F., Sahile, S. and Raja, N., 2013. Isolation and characterization of potential antibiotic producing actinomycetes from water and sediments of Lake Tana, Ethiopia. ***Asian Pacific Journal of Tropical Biomedicine***, 3(6):426-435.

Gupta, K.K., Aneja, K.R. and Rana, D., 2016. Current status of cow dung as a bioresource for sustainable development. ***Bioresources and Bioprocessing***, 3(1):1-11.

Gupta, K.K., Rana, D., 2016a. Antimicrobial Activity of Certain Bacterial Isolates–A Screening Study. ***Biotechnology International***, 9(3):55-59.

Gupta, K.K., Rana, D., 2016b. Isolation and evaluation of cow dung bacteria for their antimicrobial potential. ***Biotechnology International***, 9(2):47-54.

Harvey, A.L., 2008. Natural products in drug discovery. ***Drug Discovery Today***, 13(19-20):894-901.

Holt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST. 1994. *Bergey's Manual of Determinative Bacteriology*. 9th ed. Williams and Wilkins Press: USA;

Hsiao, A., Toy, T., Seo, H.J. and Marks, F., 2016. Interaction between Salmonella and Schistosomiasis: A Review. ***PLoS Pathogens***, 12(12):1-12.

Ilic, S.B., Konstantinovic, S.S., Todorovic, Z.B., Lazic, M.L., Veljkovic, V.B., Jokovic, N. and Radovanovic, B.C., 2007. Characterization and antimicrobial activity of the bioactive

metabolites in *streptomycete* isolates. ***Microbiology***, 76(4):421-428.

Isono, F., Takeuchi, M., Katayama, T., Seno, A., Shiozawa, H., Inukai, M., Ishii, A., Kodama, K., Haruyama, H., Watanabe, T. and Kinoshita, T., 1993. A new antibiotic, B-1015, produced by *Alcaligenes faecalis*. ***Annu Rep Sankyo Res Lab***, 45:113-118.

Kamigiri, K., Suzuki, Y., Shibazaki, M., Morioka, M., Suzuki, K.I., Tokunaga, T., Setiawan, B. and Rantiatmodjo, R.M., 1996. Kalimantacins A, B and C, Novel Antibiotics from *Alcaligenes* sp. YL-02632S. ***The Journal of Antibiotics***, 49(2):136-139.

Kapley, A., Tanksale, H., Sagarkar, S., Prasad, A.R., Kumar, R.A., Sharma, N., Qureshi, A. and Purohit, H.J., 2016. Antimicrobial activity of *Alcaligenes* sp. HPC 1271 against multidrug resistant bacteria. ***Functional & Integrative Genomics***, 16(1):57-65.

Kleinkauf, H., and von Döhren, H., 1990. *Review Nonribosomal biosynthesis of peptide antibiotics*. In EJB Reviews, Springer, Berlin, Heidelberg:151-165.

Lauková, A., Czikková, S., Vasilková, Z., Juriš, P. and Mareková, M., 1998. Occurrence of bacteriocin production among environmental enterococci. ***Letters in Applied microbiology***, 27(3):178-182.

Lehr, N.A., Meffert, A., Antelo, L., Sterner, O., Anke, H. and Weber, R.W., 2005. Antiamoebins myrocin B and the basis of antifungal antibiosis in the coprophilous fungus *Stilbella erythrocephala* (syn. *S. fimetaria*). ***FEMS Microbiology Ecology***, 55(1):105-112.

Li, Z., Peng, C., Shen, Y., Miao, X., Zhang, H. and Lin, H., 2008. L, L-Diketopiperazines from *Alcaligenes faecalis* A72 associated with South China Sea sponge *Stelletta tenuis*. ***Biochemical Systematics and Ecology***, 36(3):.230-234.

Li, Z.Y., Hu, Y., Huang, Y.Q. and Huang, Y., 2007. Isolation and phylogenetic analysis of the biologically active bacteria associated with three South China Sea sponges. ***Microbiology***, 76(4):494-499.

Liaw, S.H., Chen, S.J., Ko, T.P., Hsu, C.S., Chen, C.J., Wang, A.H.J. and Tsai, Y.C., 2003. Crystal Structure of d-Aminoacylase from *Alcaligenes faecalis* DA1 a novel subset of amidohydrolases and insights into the enzyme mechanism. ***Journal of Biological Chemistry***, 278(7):.4957-4962.

Maré, I.J. and Coetzee, J.N., 1964. Antibiotics of *Alcaligenes faecalis*. ***Nature***, 203(4943):430-431.

Mohseni, M., Norouzi, H., Hamedi, J. and Roohi, A., 2013. Screening of antibacterial producing actinomycetes from sediments of the Caspian Sea. ***International Journal of Molecular and Cellular Medicine***, 2(2):64-71.

Morgavi, D.P., Forano, E., Martin, C. and Newbold, C.J., 2010. Microbial ecosystem and methanogenesis in ruminants. ***Animal***, 4(7):1024-1036.

Nene, Y.L., 1999. *Seed health in ancient and medieval history and its relevance to present-day agriculture*. Asian Agri-History (India).

Pelaez, F., 2006. The historical delivery of antibiotics from microbial natural products—can history repeat?. ***Biochemical Pharmacology***, 71(7):981-990.

Puah, S.M., Chua, K.H. and Tan, J.A.M.A., 2016. Virulence factors and antibiotic susceptibility of *Staphylococcus aureus* isolates in ready-to-eat foods: detection of *S. aureus* contamination and a high prevalence of virulence genes. ***International Journal of Environmental Research and Public Health***, 13(2):199.

Randhawa, G.K. and Kullar, J.S., 2011. Bioremediation of pharmaceuticals, pesticides, and petrochemicals with gomeya/cow dung. ***ISRN pharmacology***, 2011: 1-7.

Shrivastava, S. and Pal, A.M.A., 2014. Cow Dung-A Boon for Antimicrobial Activity. ***Life Sciences Leaflets***, 55(2014):60-63

Teo, K.C. and Teoh, S.M., 2011. Preliminary biological screening of microbes isolated from cow dung in Kampar. ***African Journal of Biotechnology***, 10(9), pp.1640-1645.

Tripathi, C.K.M., Bihari, V. and Tyagi, R.D., 2000. Microbial production of D-amino acids. ***Process Biochemistry***, 35(10):1247-1251.

Uppal, B., Mehra, B., Panda, P.S. and Kumar, S.K., 2017. Antimicrobial susceptibility profile of *Vibrio cholerae* strains isolated at a tertiary care medical centre in New Delhi, India. ***International Journal Of Community Medicine And Public Health***, 4(3):868-871.

Wang, G.X., Li, F.Y., Cui, J., Wang, Y., Liu, Y.T., Han, J. and Lei, Y., 2011. Immunostimulatory Activities of a Decapeptide Derived from *Alcaligenes faecalis* FY-3 to Crucian Carp. ***Scandinavian Journal of Immunology***, 74(1):14-22.

Waziri, M. and Suleiman, J.S., 2012. Physicochemical properties and antimicrobial activity of evaporated extract of cow dung against some pathogens. ***Journal of Scientific Research***, 5(1):135-141.

Zahir, I., Houari, A., Bahafid, W., Iraqui, M. and Ibnsouda, S., 2013. A novel *Alcaligenes faecalis* antibacterial-producing strain isolated from a Moroccan tannery waste. ***African Journal of Microbiology Research***, 7(47):5314-5323.