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Research Article
Potential of Herbal Antibacterials as an Alternative to Antibiotics for Multiple Drug Resistant Bacteria: An Analysis

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Abstract

Background and Objective: The widespread occurrence of antimicrobial drug-resistance in pathogens is an imminent problem. The herbal antimicrobials have shown promising potential and are often relied upon as a potential alternative to antibiotics. The present study aimed at determining drug resistance trends and evaluates the potential of herbal antimicrobials to combat multi-drug-resistant bacterial infections. **Materials and Methods:** The 11 year (2009-2019) data available at Clinical Epidemiology Laboratory of Indian Veterinary Research Institute, Izatnagar on susceptibility for antibiotics (70) and herbal antimicrobials (26) for 6171 bacteria from clinical (3515) and non-clinical (2656) samples were analyzed to estimate the efficacy of antibiotics and herbal antimicrobials and their correlations. **Results:** The analysis revealed an upward trend of resistance against almost all antibiotics except ampicillin. Antimicrobial resistance towards carbapenems was more often detected in bacteria isolated from clinical cases than those from non-clinical samples. The efficacy of herbal antimicrobials on multiple drug-resistant strains was limited. The zone of bacterial growth inhibition around antibiotic and herbal antimicrobial discs had a positive correlation ($r \geq 0.027$, $p \leq 0.01$) between the two. Multiple antibiotic resistance indices and multiple herbal antimicrobial resistance indices also had a strong correlation ($r \geq 0.048$, $p \leq 0.01$). **Conclusion:** Analysis indicated that herbal drug antimicrobial resistance and antibiotic resistance might go hand in hand. Thus, herbal antimicrobials may not be seen as an alternative to antibiotics for the treatment of infections caused by multiple-drug-resistant bacteria in animals. However, herbal antimicrobials may be alternatives to antibiotics as antibiotics are alternatives to each other.

Key words: Alternative medicine, MDR, carbapenem-resistance, herbal antimicrobial resistance, clinical-infections, antimicrobial drug resistance index, ajowan oil, cinnamon oil, carvacrol, citral

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antimicrobial drug resistance (AMR) making most of the antibiotics useless in therapeutics is a global trend and everyone is worried about the future of antibiotics as an effective therapy to counter bacterial infections[1].Though AMR exists from the pre-antibiotic era, the use and misuse of antibiotics accelerated its spread[2]. The AMR is not the only problem in medical practice but a global problem and it has been emphasized to be seen from the perspective of One World One health[3]. The eminent threat limiting the utility of antibiotics rushed scientists for the search of alternatives to antibiotics and herbal antimicrobials are seen as the first-hand help[4]. In many parts of the world, herbal compounds or preparations are looked at as potential alternatives to antibiotics for long[4]. A WHO report reviewing traditional medicine uses in developing countries emphasized the need to define the role of traditional herbal cures in alternative and complementary medicine by developing a strategy to address issues of policy, safety, efficacy, quality, access and rational use of traditional, complementary and alternative medicine[5]. Several researchers have seen the future in herbal antimicrobials to combat antimicrobial-resistant (AMR) microbes[6-8]. However, a few authors selectively criticized the idea of herbal antimicrobial compounds as a useful alternative for antibiotics[9] and suggested other alternates either specific as bacteriophage therapy, bacteriocins, predatory bacteria and vaccines or methods of general utility as competitive exclusion, immunotherapeutics, prebiotics, probiotics and synbiotics[10, 11]. Nowadays herbal antimicrobials are a hot spot of research and every year hundreds of research papers are published. However, instead of increasing lucidity as expected by WHO more than 17 years ago[5], the pouring researches are creating more confusion.

Thus this analysis of pre-existing antimicrobial and herbal antimicrobial sensitivity assay data on more than 6000 bacteria isolated from veterinary clinical and non-clinical samples was conducted to understand trends of antimicrobial drug resistance and the potential of herbal antimicrobials for combating the bacteria with AMR and multi-drug-resistance.

MATERIALS AND METHODS

Study area: The study was carried out at Division of Epidemiology, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India from 2009 to 2019.

Data collection: The data for sensitivity (inhibition zones measured in mm) of bacterial isolates from clinical cases (treated in the various veterinary hospitals in and around Bareilly) against 70 antibiotics (amdinocillin, amikacin, amoxicillin+clavulanic acid, amoxicillin, amoxicillin+ sulbactam, ampicillin+sulbactam, ampicillin, ampicillin+ cloxacillin, azithromycin, aztreonam, bacitracin, cefdinir, cefepime, cefepime+tazobactam; cefixime, cefoperazone, cefotaxime, cefotaxime+clavulanic acid, cefoxitin, cefoxitin+ cloxacillin, cefpodoxime, ceftriaxone+ sulbactam, ceftazidime, ceftazidime+ clavulanic acid, ceftriaxone, ceftriaxone+ tazobactam, cephalexin, cefoperazone+ sulbactam, chloramphenicol, ciprofloxacin, clindamycin, cloxacillin, colistin, cotrimoxazole, doxycycline, ertapenem, erythromycin, fosfomycin, gatifloxacin, gentamicin, imipenem, kanamycin, lincomycin, linezolid, meropenem, methicillin, minocycline, moxalactam, mupirocin, nalidixic acid, netilmicin, nitrocefin, nitrofurantoin, norfloxacin, novobiocin, oxacillin, oxytetracycline, penicillin, piperacillin, piperacillin tazobactam, polymyxin B sulphate, spectinomycin, streptomycin, teicoplanin, tetracycline , tigecycline, trimethoprim, tylosin, vancomycin and virginiamycin) was used in the study. However, antimicrobial resistance trends were analyzed for only amoxicillin, ampicillin, azithromycin, aztreonam, ceftriaxone, chloramphenicol, ciprofloxacin, colistin, cotrimoxazole, gentamicin, imipenem, nitrofurantoin, penicillin, piperacillin+tazobactam, tetracycline and tigecycline was retrieved from Clinical Epidemiology Laboratory of Indian Veterinary Research Institute, Izatnagar database. Besides, for herbal antimicrobials sensitivity results available against one mg discs of 26 herbal antimicrobials including agarwood (*Aquilaria malaccensis*) oil, *methanolic Ageratum conyzoides* leaves, ajowan (*Tachyspermum ammi*) oil, *Artemesia vulgaris* essential oil, betel (*Piper betel*) leaf oil, caraway (*Carum carvi*) oil, carvacrol, cinnamaldehyde, cinnamon (*Cinnamomum verum*) oil , citral, citronella oil, methanolic extract of *Eupatorium conyzoides* leaves, eucalyptus (*Eucalyptus globulus*) gum, guggul (*Commiphora wightii*) oil, holy basil (*Ocimum sanctum*)oil, methanolic extract of Kalonji (*Nigella sativa*) seeds, lemongrass (*Cymbopogon citrates*) oil, marjoram (*Origanum majorana*) essential oil; *Zanthoxylum rhetsa* seed carp essential oil, methanolic extract of *Zanthoxylum rhetsa* seed carp, oil of *Zanthoxylum rhetsa* seeds, patchouli (*Pogostemon cablin*) essential oil, rose geranium (*Pelargonium graveolens*) essential oil, rosewood (*Dalbergia sissoo*) essential oil, sandalwood (*Santalum album*)oil, thyme (*Thymus vulgaris*) oil and tea tree (*Melaleuca alternifolia*) oil. Antimicrobial resistance trends were analyzed for ajowan oil, betel leaf oil, carvacrol, cinnamaldehyde, cinnamon oil, citral, guggul oil, holy basil oil,

lemongrass oil, sandalwood oil and thyme oil was also retrieved from Clinical Epidemiology Laboratory of Indian Veterinary Research Institute, Izatnagar database.

Methodology: Data on antimicrobial sensitivity (herbal antimicrobials as well as conventional antimicrobials and antibiotics) retrieved from the database of Clinical Epidemiology Laboratory of ICAR-Indian Veterinary Research Institute, Izatnagar, was transferred to Microsoft Office Excel worksheet. Data of only those isolates having been tested for one or more herbal antimicrobials along with antibiotics was included in the analysis and grouped to analyze. Bacterial isolates were classified as resistant or sensitive based on inhibition zone measured in mm as per guidelines of CLSI where so ever applicable[12,13]. For herbal antimicrobial discs, the inhibition zone, if any around the disc, was interpreted as an indicator of the sensitivity of the bacteria to the tested herbal antimicrobial and inhibition zones were recorded in mm as described earlier[14].

Multiple antimicrobial drug resistance index (MARI= number of antimicrobial drugs resisted/number of antimicrobial drugs tested) and multiple herbal antimicrobial drug resistance index (MHARI= number of herbal antimicrobial drugs resisted/number of herbal antimicrobial drugs tested) were calculated for each strain as described earlier[14].

Statistical analysis: Data were analyzed using statistical tools like Pearson correlation odds ratio, Chi-square/Fisher's exact test.

RESULTS AND DISCUSSION

The present study analyzed 11-year (2009-2019) data available in Clinical Epidemiology of Indian Veterinary Research Institute, Izatnagar, on an antibiotic (70, tested as per requirement and CLSI recommendations) and herbal antimicrobial (26) sensitivity of bacteria belonging to 68 genera (6171, Table 1) isolated from clinical cases (3515, belonging to 58 genera) and non-clinical samples (2656, belonging to 46 genera).

The correlation analysis of the zone of bacterial growth inhibition around antibiotic and herbal antimicrobial discs revealed a strong positive correlation (p, 0.05). Further, multiple antibiotic resistance indices (MARI) and multiple herbal antimicrobial resistance indices (MHARI) of bacteria also had a good positive correlation ($p \leq 0.01$) with each other. Analysis indicated that herbal drug antimicrobial resistance and antibiotic resistance go hand in hand as suggested earlier on studies on a limited number of strains[15-17]. It may be concluded from the analysis that herbal antimicrobials may not be seen as an alternative to antibiotic for the treatment of infections caused by Multiple-Drug-Resistant (MDR), Carbapenem-Resistant (CR) and Extended-Spectrum-β-Lactamase (ESBL) producer bacteria ($p \leq 0.01$) but maybe effective only in some of the cases.

The ESBL producer strains were more often susceptible to colistin, polymyxin B, minocycline, cephalosporins with ESBL inhibitors (clavulanic acid, sulbactam, tazobactam) and carbapenems ($p \leq 0.01$). It indicated the potential of these less used antibiotics instead of herbal antimicrobials for the treatment of infections with ESBL producer strains.

Except for eucalyptus gum ($p \leq 0.05$), none of the herbal antimicrobials was more active on MDR strains than on non-MDR strains; however, it acted only on 9.8% of the isolates tested. Though no herbal antimicrobial had the potential to beat MDR strains, ciprofloxacin, cotrimoxazole, gentamicin, nalidixic acid, norfloxacin, streptomycin and tetracycline were more effective on multiple herbal antimicrobial-resistant (MHAR) strains than on non-MHAR bacteria ($p \leq 0.01$). The analysis revealed that some of the herbal antimicrobials may be very good and even better than some of the antibiotics in their spectrum of antibacterial activity but they can't be considered as an alternative to treat infection caused by MDR strains, being ineffective or not more effective than conventional antibiotics on MDR strains. However, herbal antimicrobials may be a potential antibiotic alternative in the way similar to one antibiotic is for the other(s).

Antibiotic sensitivity data on 70 antibiotics revealed that only nine antibiotics (cefoperazone+sulbactam, 94.34%; tigecycline, 92.08%; imipenem, 91.42%; cefepime +tazobactam, 90.13%; ceftriaxone+tazobactam, 87.93%; chloramphenicol, 85.01%; ceftriaxone+sulbactam, 84.72%; meropenem, 84.72% and netilmicin, 82.56%) could inhibit growth of more than 80% of the isolates causing clinical infections in animals and birds. On the other hand, of the 26 herbal antimicrobials, tested using disc (containing 1 mg of the active component) diffusion assay, only five (carvacrol, 96.66%; ajowan oil, 93.45%; cinnamaldehyde, 93.45%; thyme oil, 91.74 and cinnamon oil, 90.56%) inhibited >80% of the isolates. Similar efficacy of the herbal antimicrobials has been indicated in earlier studies[15,18-20].

Table 1: Antimicrobial and herbal antimicrobial resistance in bacterial isolates from clinical (C) and non clinical (NC) samples at Clinical Epidemiology Laboratory since 2009 till 2019

Genus of bacteria tested	Source of isolation	Total isolates tested	Carbapenem resistant	Multiple drug resistant	ESBL producers	MARI	MHARI
Achromobacter	NC 1, C9	10	3	7	7	0.51	0.64
Acinetobacter	NC 49	49	31	37	20	0.53	0.43
Acinetobacter	C 55	55	20	36	31	0.40	0.33
Actinobacillus	NC 1, C 8	9	3	6	6	0.40	0.45
Actinomycess	C 5	5	3	5	2	0.59	0.31
Aerococcus	NC 6	6	1	3	0	0.40	0.36
Aerococcus	NC 33	33	6	20	15	0.34	0.31
Aeromonas	C 137	137	30	70	100	0.36	0.37
Aeromonas	NC 97	97	33	55	44	0.40	0.37
Aggregatibacter	C 3	3	0	0	2	0.21	0.34
Agrobacterium	NC1, C5	6	0	4	1	0.39	0.60
Alcaligenes	NC 30	30	16	24	15	0.54	0.45
Alcaligenes	C 63	63	16	45	32	0.38	0.43
Arsenophonus	C 3	3	0	1	1	0.28	0.17
Avibacterium	C 8	8	1	1	0	0.24	0.16
Bacillus	NC 80	80	6	32	22	0.29	0.22
Bacillus	C 77	77	5	37	40	0.30	0.33
Bordetella	NC 9	9	1	7	7	0.47	0.56
Bordetella	C 16	16	2	4	3	0.27	0.34
Branhamella	C 1	1	0	1	0	0.29	0.11
Brevibacillus	C 1	1	0	1	1	0.20	0.17
Budvicia	C 5	5	1	3	1	0.46	0.29
Burkholderia	NC 4, C 14	18	2	7	7	0.33	0.52
Campylobacter	NC 4	4	0	0	0	0.25	1.00
Chryseomonas	C 1	1	1	1	0	0.76	0.15
Citrobacter	NC 187	187	1	9	15	0.17	0.80
Citrobacter	C 27	27	3	4	19	0.26	0.43
Corynebacterium	C 5	5	0	1	2	0.25	0.21
Cytophaga	NC 1	1	0	1	1	0.33	0.00
Dermatophilus	C 4	4	0	0	2	0.13	0.52
Edwardsiella	NC 44	44	3	11	5	0.22	0.58
Edwardsiella	C 20	20	2	7	12	0.28	0.52
Enterobacter	NC 175	175	27	69	44	0.30	0.59
Enterobacter	C 212	212	22	110	121	0.38	0.49
Enterococcus	NC 301	301	55	96	19	0.30	0.76
Enterococcus	C 73	73	24	50	30	0.46	0.49
Erwinia	NC 81	81	9	33	52	0.29	0.37
Erwinia	C 45	45	6	26	32	0.35	0.45
Escherichia	NC 530	530	71	178	112	0.29	0.55
Escherichia	C 1001	1001	122	634	611	0.42	0.48
Ewingella	NC 2	2	0	0	0	0.09	1.00
Flavimonas	NC 1	1	1	1	0	0.96	0.82
Flavobacterium	C 9	9	0	7	3	0.35	0.23
Gallibacterium	C 29	29	0	14	7	0.24	0.27
Geobacillus	NC 1, C 2	3	0	1	1	0.26	0.50
Gordonia	C 1	1	0	1	0	0.56	0.83
Haemophilus	C 1	1	1	0	1	0.36	0.42
Hafnia	NC 8	8	2	2	1	0.26	0.59
Hafnia	C 19	19	1	15	15	0.44	0.49
Klebsiella	NC 214	214	24	72	36	0.28	0.66
Klebsiella	C 141	141	12	98	83	0.44	0.49
Kluyvera	NC 4, C 3	7	1	2	1	0.20	0.62
Leclercia	NC 2	2	0	0	0	0.00	0.75
Leminirella	NC 2, C 1	3	0	0	1	0.16	0.58
Listeria	NC 1	1	0	0	1	0.08	0.38
Listonella	NC 3	3	0	0	0	0.28	0.22

Table 1: Continue

Genus of bacteria tested	Source of isolation	Total isolates tested	Carbapenem resistant	Multiple drug resistant	ESBL producers	MARI	MHARI
Micrococcus	NC 13	13	0	4	4	0.30	0.37
Micrococcus	C 43	43	9	23	20	0.32	0.30
Moraxella	NC 7	7	2	2	6	0.22	0.27
Moraxella	C 38	38	6	15	17	0.31	0.35
Morganella	C 3	3	1	3	3	0.53	0.44
Obesumbacterium	C 1	1	0	1	1	0.38	0.50
Paenibacillus	C 1	1	0	0	1	0.12	0.00
Pasteurella	NC 12	12	0	1	10	0.17	0.71
Pasteurella	C 41	41	1	8	26	0.22	0.39
Plesiomonas	C 5	5	0	0	0	0.21	0.63
Pragia	NC 24	24	1	5	0	0.20	0.87
Pragia	C 5	5	1	2	1	0.32	0.75
Prdiococcus	C 1	1	0	1	1	0.39	0.88
Proteus	NC 32	32	6	15	7	0.32	0.56
Proteus	C 122	122	30	98	66	0.50	0.53
Providencia	NC 8	8	2	5	0	0.53	0.46
Providencia	C 5	5	2	4	3	0.47	0.45
Pseudomonas	NC 103	103	39	75	31	0.50	0.56
Pseudomonas	C 167	167	57	128	81	0.56	0.70
Raoultella	NC 24	24	2	8	4	0.28	0.59
Raoultella	C 38	38	10	22	29	0.43	0.50
Roseomonas	C 1	1	1	1	0	0.81	0.42
Salmonella	NC 216	216	1	4	6	0.20	0.82
Salmonella	C 37	37	2	12	14	0.30	0.65
Serratia	NC 47	47	8	17	12	0.30	0.51
Serratia	C 28	28	3	17	18	0.41	0.39
Shewanella	NC 3, C3	6	5	5	3	0.48	0.33
Sphingomonas	C 2	2	0	0	2	0.23	0.71
Staphylococcus	NC 250	250	43	135	85	0.32	0.47
Staphylococcus	C 530	530	86	307	240	0.33	0.36
Stomatococcus	C 1	1	0	0	0	0.15	0.50
Streptobacillus	C 1	1	0	0	0	0.00	0.29
Streptococcus	NC 56	56	13	31	4	0.41	0.71
Streptococcus	C 345	345	72	182	92	0.33	0.44
Vibrio	NC 9	9	0	1	0	0.22	0.14
Vibrio	C 9	9	1	4	5	0.33	0.45
Xanthomonas	NC 1	1	0	1	1	0.38	0.08
Xenorhabdus	NC 6	6	2	2	2	0.24	0.40
Xenorhabdus	C 10	10	1	8	4	0.37	0.46
Yersinia	NC 1	1	0	0	0	0.31	1.00

ESBL: Extended spectrum β-lactamase; MARI: Multiple antibiotic resistance index, MHARI: Multiple herbal antimicrobial resistance index, carbapenem resistant, resistant to one or more drug of carbapenem family including meropenem, Imipenem and ertapenem

Except for guggul oil and ampicillin, resistance trends for antibiotics and herbal antimicrobial had an upward trend (Table 2). It might be due to the continued existence of the selection pressure induced by use or misuse antimicrobials and polluting the environment[1,2,3,21] and appears to be an expected trend.

The antimicrobial sensitivity of non-clinical isolates was quite different than clinical isolates. Instead of nine antibiotics, >80% of bacteria from non-clinical samples were inhibited by 18 antimicrobials (netilmicin, 96.21%; cefoperazone+ sulbactam, 93.62%; kanamycin, 93.47%; gatifloxacin, 92.69%, cefoxitin-cloxacillin, 92.23%; ceftriaxone+tazobactam, 91.53%, ceftriaxone+sulbactam, 90.00%; tigecycline, 89.67%; chloramphenicol, 88.94%; gentamicin, 86.91%; streptomycin, 86.70%; minocycline, 85.45%; norfloxacin, 84.11%; imipenem, 83.45%; ciprofloxacin, 83.00%; doxycycline, 80.86%; oxytetracycline, 80.47% and amikacin, 80.11%). It indicated that bacteria causing clinical infection have a better range of antimicrobial resistance potential than those from non-clinical samples. However, five best herbal antimicrobials for non-clinical and clinical isolates were the same but less number of non-clinical isolates were sensitive to herbal antimicrobials

Table 2: Important antimicrobial resistance trend in veterinary clinical isolates of bacteria isolated from diseased animals and birds

	Percent resistant strains in different year											
Antimicrobial tested	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Average
Amoxicillin	46.81	48.81	51.81	54.19	60.66	70.05	62.24	60.29	51.22	58.03	58.18	56.57
Ampicillin	61.61	62.01	56.61	54.92	74.56	72.66	63.08	60.61	52.7	60.03	51.28	60.92
Azithromycin	24	25.5	25.8	27.93	34.7	39.32	53.99	48.66	48.87	50.67	52.38	39.26
Aztreonam	38.03	42.03	46.03	48.86	56.48	69.06	70.44	73.2	76.21	68.92	68	59.75
Ceftriaxone	18.27	19.77	20.87	23.89	24.94	25.41	29.62	32.74	33.1	29.49	27.81	25.99
Chloramphenicol	9.87	10.88	11.89	15.07	21.01	18.09	13.76	14.46	18.01	17.68	14.21	14.99
Ciprofloxacin	22.43	23.93	24.93	25.94	34.93	39.92	40.94	47.75	46.82	45.7	42.53	35.98
Colistin	30.08	30.28	30.38	31.17	34.26	35.99	37.79	37.6	37.76	37.25	44.13	36.15
Cotrimoxazole	33.95	34.65	36.85	39.98	45.43	42.78	44.95	46.68	51.53	52.54	54.18	43.96
Gentamicin	25.03	25.93	23.43	19.03	14.71	13.38	36.26	27.27	31.3	29.68	23.47	24.5
Imipenem	1.28	2.08	3.28	4.55	5.67	6.28	7.89	10.8	12.73	18.34	21.47	8.58
Nitrofurantoin	26.21	27.02	27.22	22.26	26.17	26.86	26.99	23.46	22.1	29.82	20.62	25.34
Penicillin	62.22	65.02	63.02	68.17	85.7	67.65	86.31	67.73	59.73	51.95	60	67.04
Piperacillin + Taztobactam	23.33	23.43	24.53	26.1	28.11	25.47	24.35	21.8	24.02	15.12	21.99	23.48
Tetracycline	29.93	33.93	35.73	38.8	40.09	42.19	41.61	53.51	50.84	54.45	71.88	45.81
Tigecycline	5.36	6.46	7.26	11.54	9.46	7.8	7.39	5.51	10.65	6.38	9.29	7.92
Multiple drug resistance	46.11	48.98	49.11	58.18	61.08	62.82	66.3	64.94	66.31	64.77	60.87	59.04
Herbal antimicrobials												
Ajowan oil	2.08	2.68	2.6	4.02	4.58	6.25	4.28	5.29	7.98	6.68	14.63	6.55
Betel leaf oil	32.07	33.42	32.02	32	33	31.68	33.28	40.21	34.08	25.07	69.27	36.01
Carvacrol	1.01	1.32	1.2	2.88	3.99	3.45	2.82	2.66	3.82	3.78	9.76	3.34
Cinnamledehyde	4.71	4.8	4.91	4.95	5.7	5.9	6.38	6.91	7.05	8.74	11.95	6.55
Cinnamon oil	5.17	5.27	5.67	6.7	7.98	8.76	7.39	7.36	8.97	12.24	28.29	9.44
Citral	35.2	40.3	43.2	59	62	60.73	44.16	52.5	41.32	45.8	51.96	48.74
Guggul oil	65.92	75.32	81.92	85.02	85.71	84.25	77.94	81.36	72.46	69.61	70.73	77.29
Holy basil oil	16.88	17.38	8.08	20.7	24.07	22.14	19.85	17.36	17.6	17.85	29.51	20.22
Lemongrass oil	56.03	63.03	64.13	74.36	56.02	42.18	60.87	74.43	40.13	54.51	64.22	59.08
Sandalwood oil	65.13	65.93	70.93	74.97	73.94	57.39	68.89	78.13	59.84	60.81	71.57	67.96
Thyme oil	6.45	6.85	6.95	7.2	7.99	6.92	6.67	5.86	8.16	6.3	21.46	8.26
	Drug resistance indices in											
	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Overall
Multiple antibiotic resistance indices	0.3	0.4	0.4	0.41	0.46	0.38	0.41	0.35	0.39	0.38	0.41	0.39
Multiple herbal antimicrobial resistance indices	0.3	0.4	0.58	0.68	0.61	0.46	0.42	0.46	0.31	0.31	0.45	0.45

than clinical isolates viz., carvacrol, 93.52%; ajowan oil, 91.84%; thyme oil, 88.64%; cinnamaldehyde, 84.97% and cinnamon oil, 82.09%. It might be due to the survival strategy of bacteria in an environment full of different kinds of vegetation in the area of study.

Comparison of sensitivity patterns of bacteria from clinical and non-clinical samples for most commonly used antibiotics and effective herbal antimicrobials revealed that bacteria associated with clinical infections were significantly more often ($p<0.01$) resistant to chloramphenicol, netilmicin, cefoxitin+cloxacillin, gentamicin, ciprofloxacin, cotrimoxazole and tetracycline than isolates from non-clinical samples.

For most of the herbal antimicrobials, there was a significant difference ($p\leq0.02$) for the sensitivity of bacteria from clinical and non-clinical sources except for ajowan oil (p, 0.09), holy basil oil (p, 0.1) and citral (p, 0.49). It might be due to the high efficacy of ajowan against most of the bacteria[18]. Except for methanolic extract of *A. conyzoides* Kalonji seeds and tea tree oil, clinical isolates had lesser odds (≤0.8) of being resistant to other herbal antimicrobials (carvacrol, thyme oil, cinnamaldehyde, cinnamon oil, betel leaf essential oil, rosewood oil and lemongrass oil) than bacteria from non- clinical sources ($p\leq0.01$). Among the most commonly isolated bacteria associated with clinical infections, there was no significant difference with respect to carbapenem resistance (CR), MDR and ESBL production by *Staphylococcus* spp. isolates. However, CR was more often recorded in clinical isolates of *Acinetobacter* sp. (3.01; p, 0.007), *Aeromonas* spp. (1.97; p, 0.03); *Alcaligenes* spp. (3.54; p, 0.006), *Enterobacter* spp. (2.43; p, 0.004), *Enterococcus* spp. (4.11; p, <0.001), *Escherichia* spp. (1.53; p, 0.01), *Klebsiella* spp. (2.73; p, 0.007) and *Streptococcus* spp. (3.18; p, 0.004) than in isolates of the same group of bacteria from non-clinical sources. Bacterial isolates of *Citrobacter* spp. (0.29; p, 0.04),

Enterobacter spp. (0.60; p, 0.014), *Enterococcus* spp. (0.22; p<0.001), *Escherichia* spp. (0.29; p<0.001), *Klebsiella* spp. (0.22; p<0.001), *Proteus* spp. (0.22; p, <0.001), *Salmonella* spp. (0.04; p, <0.001) and *Serratia* spp. (0.38; p, 0.039) from clinical samples had significantly lesser odds of being MDR type than their siblings from non-clinical samples. Similarly, ESBL production by clinical isolates of *Bacillus* spp. (0.42; p, 0.012), *Edwardsiella* spp. (0.24; 0.0.03), *Enterobacter* spp. (0.49; p, 0.002), *Enterococcus* spp. (0.43; p, 0.017), *Escherichia* spp. (0.32; p<0.001), *Pseudomonas* spp. (0.54; p, 0.02), *Raoultella* spp. (0.18; p, 0.013) and *Salmonella* spp. (0.33; p, 0.043) was significantly less common than among their siblings from non-clinical samples. Somewhat similar variations have been reported earlier for certain herbal antimicrobials[14,22,23] and antibiotics[24,25] indicating a higher prevalence of MHDR and MDR strains in the environment than those causing clinical infections. A load of MDR and ESBL producing bacteria in the environment indicated that potentially pathogenic strains with high antimicrobial resistance might be all around us.

The analysis also revealed the most common to the rarely isolated bacteria associated with clinical infections in veterinary practice (Table 1). The 20 most common genera of bacteria associated with clinical infections in their decreasing frequency were, *Escherichia, Staphylococcus, Streptococcus, Enterobacter, Pseudomonas, Klebsiella, Aeromonas, Proteus, Bacillus, Enterococcus, Alcaligenes, Acinetobacter, Erwinia, Micrococcus, Pasteurella, Moraxella, Raoultella, Salmonella, Gallibacterium, Serratia* and *Citrobacter* species. Many of them have already been reported common in animals in other parts of the world too and are of zoonotic potential[26]. Therefore, the increasing trend for AMR, MDR and MHDR in bacteria from veterinary clinical and animal environmental samples are not only of serious concern for animal husbandry, livestock and pet owners but to whole humans' society. Spread of AMR bacteria from animals to humans is not an imaginary threat but well-proven fact[27,28]. The observations of the study may not only help the veterinarians to think of the probable causes of common diseases in animals and birds and to decide a more effective therapeutic line judiciously using the AMR trends but also to the medical community and other microbiologists.

The study summarized that herbal antimicrobial drug resistance and antibiotic resistance are concurrent in bacteria. The herbal antimicrobials were as prone to the development of resistance as the antibiotics and herbal antimicrobials have no big advantage towards MDR strains of bacteria. Some of the herbal antimicrobials may be good alternatives to antibiotics but in the way similar to one antibiotic is to the other.

CONCLUSION

The study concludes that some of the herbal antimicrobials (cinnamon oil, cinnamaldehyde, carvacrol, ajowan oil, thyme oil) may be very effective against bacteria but certainly not be the alternative to antibiotics to cure infection with MDR strains of clinically important bacteria.

SIGNIFICANCE STATEMENT

This study analyzed the trends of antimicrobial drug resistance and compared the efficacy of herbal antimicrobials and conventional antibiotics on clinical and non-clinical bacterial isolates. The study revealed that AMR trends are on a steady increase and some of the herbals (cinnamon oil, cinnamaldehyde, carvacrol, ajowan oil, thyme oil) have potential as broad-spectrum antimicrobials. The study indicated that herbal antimicrobials are no more effective on multiple-drug-resistant (MDR) strains than on non-MDR strains. This study will help all stakeholders of antimicrobial therapy including the researcher to uncover the critical gaps in areas of new antimicrobial drug development, pharmaceuticals to develop suitable herbal antimicrobials and clinicians to judiciously use the herbal as well as conventional antimicrobials in therapeutics.

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REFERENCES

1. Davey, P., M.H. Wilcox, W. Irving and G. Thwaites, 2015. Antimicrobial Chemotherapy. 7th Edn., Oxford University Press, United Kingdom, Pages: 432.
2. Chellat, M.F., L. Raguž and R. Riedl, 2016. Targeting antibiotic resistance. Angew. Chem. Int. Ed., 55: 6600-6626.
3. Singh, B.R., 2019. One health –anti microbial resistance and its impact on human & animal health–veterinary perspective. In: Conference proceedings: One Health – National Conference on Anti Microbial Resistance & Prevention of Diseases of Public Health Importance (Zoonotic Diseases), Feb. 2019, Mumbai, India. -.
4. Chandra, H., P. Bishnoi, A. Yadav, B. Patni, A.P. Mishra and A.R. Nautiyal, 2017. Antimicrobial resistance and the alternative resources with special emphasis on plant-based antimicrobials-A review. Plants, Vol. 6. 10.3390/plants6020016

5. World health organization, 2002. WHO traditional medicine strategy 2002-2005. Geneva, Pages: 61.
6. Basappa, K, and J. Venugopal, 2013. Natural alternatives to antibiotic agents. Asian J. Biomed. Pharmaceut. Sci.,
7. Ionescu, M.I., 2017. Are herbal products an alternative to antibiotics? Bact. Pathogen. Antibact. Cntrl. Sahra, 10.5772/intechopen.72110
8. Buhner, S.H., 2012. Herbal antibiotics: Natural alternatives for treating drug-resistant bacteria. 2nd Edn., Storey Publishing, USA, Pages: 480.
9. Martin, K.W. and E. Ernst, 2003. Herbal medicines for treatment of bacterial infections: A review of controlled clinical trials. J. Antimicrob. Chemother., 51: 241-246.
10. Allen, H.K., 2017. Alternatives to antibiotics: why and how. NAM Perspect., 10.31478/201707g
11. Cheng, G., H. Hao, S. Xie, X. Wang, M. Dai, L. Huang and Z. Yuan, 2014. Antibiotic alternatives: The substitution of antibiotics in animal husbandry? Front. Microbiol., Vol. 5. 10.3389/fmicb.2014.00217
12. CLSI, 2006. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria. Clinical and Laboratory Standards Institute, Wayne, PA., USA.
13. Clinical and laboratory standards Institute, 2014. Performance standards for antimicrobial disk susceptibility tests. 24th informational supplement, Document M100-S24 and M11-A8.Clinical and Laboratory Standards Institute, Wayne, Pennsylvania.Pages 249. ISBN 1-56238-805-3.
14. Singh, B.R., D.K. Sinha, O.R. Vinodh Kumar, A.M. Pawde, U.K. De and V.K. Gupta, 2019. Comparative antimicrobial activity of *Pogostemoncablin* (patchouli) essential oil (PEO) and conventional antimicrobials against clinically important microbes, World J. Pharmaceut. Sci., 7: 47-65.
15. Vadhana, P., B.R. Singh, M. Bhardwaj and S.V. Singh, 2015. Emergence of herbal antimicrobial drug resistance in clinical bacterial isolates. Pharm. Anal. Acta, Vol. 6. 10.4172/2153-2435.1000434
16. Bhardwaj M., B.R. Singh and D.K. Sinha, 2017. Potential of herbal drug and antibiotic combination therapy: a new approach to treat multidrug resistant bacteria. Pharm. Anal. Acta, 10.4172/2153-2435.1000523
17. Singh, B.R., D.K. Sinha, O.R. Vinodh Kumar, P. Vadhana and M. Bhardwaj *et al*., 2019. Potential of herbal drug and antibiotic combination therapy: a new approach to treat multidrug resistant bacteria. Cur. Drug Discov. Technol., 10.2174/1570163816666190125163536
18. Singh, B.R., V. Singh, N. Ebibeni and R.K. Singh, 2013. Antimicrobial and herbal drug resistance in enteric bacteria isolated from faecal droppings of common house lizard/gecko (*Hemidactylus frenatus*). Int. J. microbiol.,
19. Singh, B.R., D.K. Sinha and O.R.V. Kumar, 2016. Effect of herbal antimicrobials on bacterial strains of foods of vegetable and animal origin. J. Food Chem. Nanotechnol., 2: 115-123.
20. Singh, B.R., O.R. Vinodhkumar, D.K. Sinha, M. Bhardwaj and A.S.P. Vadhana, 2017. Antimicrobial resistance profile of enteropathogens isolated from diarrhea patients: Herbal antimicrobials, a ray of hope. Annals Pharmacol. Pharmaceut., 2: 1068-1078.
21. Kumar, S. and B.R. Singh, 2013. An overview on mechanisms and emergence of antimicrobials drug resistance. Adv. Ani. Vet. Sci., 1: 7-14.
22. Singh, B.R., R. Agrawal, D.K. Sinha, O.R. Vinodh Kumar and P. Vadhana *et al*., 2016. Antimicrobial activity of methanolic extract and ether extract of *Ageratum conyzoides*. Pharm. Anal. Acta, 07: 50-59.
23. Singh, B.R., V. Singh, R.K. Singh and N. Ebibeni, 2011. Antimicrobial activity of lemongrass (*Cymbopogon citratus*) oil against microbes of environmental, clinical and food origin. Int. Res. J. Pharm. Pharmacol., 1: 228-236.
24. Van Duin, D. and D.L. Paterson, 2016. Multidrug-resistant bacteria in the community: Trends and lessons learned. Infect. Dis. Clin., 30: 377-390.
25. Otokunefor, K., P. Agbude and T.V. Otokunefor, 2018. Non-clinical isolates as potential reservoirs of antibiotic resistance in Port Harcourt, Nigeria. Pan. Afr. Med. J., 10.11604/pamj.2018.30.167.14261
26. Ghasemzadeh, I. and S.H. Namazi, 2015. Review of bacterial and viral zoonotic infections transmitted by dogs. J. Med. Life.,
27. Price, L.B., J.P. Graham, L.G. Lackey, A. Roess, R. Vailes and E. Silbergeld, 2007. Elevated risk of carrying gentamicin-resistant *Escherichia coli* among u.s. poultry workers. Environ. Health Perspect., 115: 1738-1742.
28. Akwar, T.H., C. Poppe, J. Wilson, R.J. Smith and D.M. Waddington, 2007. Risk factors for antimicrobial resistance among fecal *Escherichia coli* from residents on forty-three swine farms. Microb. Drug Resist., 13: 69-76.