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Study on Multi Drug Resistant Opportunistic Pathogens Obtained from Clinical Settings of Tamil Nadu for Developing Novel Alternative Therapeutics

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Abstract

Opportunistic pathogens prevail in the hospital environment, and utensils are the root cause of severe nosocomial infection. These pathogens exhibit high antibiotic resistance due to constant exposure to drug therapy. This study focuses on screening antibiotic-resistant opportunistic pathogen and effectiveness of piperidine compounds against the opportunistic pathogens. Standard microbiological laboratory protocols were used and followed, and about 238 samples were processed and screened. Among them, 47 reported positive for the presence of pathogens like *Staphylococcus* species, *Salmonella* species, *Pseudomonas* species, *Proteus* species, *E. coli* and *Klebsiella* species. In antibiotic resistance screening, the maximum resistance percentage was recorded against Ampicillin and Chloramphenicol (100%). The least percentage of resistance was noticed against Carbenicillin (41%). Piperidine compounds showed promising susceptibility towards test isolates. The MIC of the compounds against *E. coli* and *Staphylococcus* sp. was found to be higher when compared to *Klebsiella* sp.

Keywords: opportunistic pathogens, piperidines, antibiotic resistance, nosocomial infection.

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INTRODUCTION

"Opportunistic pathogens" name tossed for the organisms which have the potential to establish infection upon obtaining the favourable situation. But in the hospital settings, opportunistic pathogens are the organism which could cause generalised disease to those patients who have a greatly diminished resistance to infection. Since the long-term use of antibiotics may alter normal flora leads to an increase in opportunistic microorganisms[1]. The patient in the post-operative ward or the immune-compromised ward is more susceptible for these opportunistic pathogens like *Staphylococcus* species, Enterobacteriaceae members and yeasts[2-4]. Here these microbes find the way by itself beyond the physical barrier and establish infection. For example, *Pseudomonas aeruginosa,* which most commonly causes burn and external infections, colonise on medical devices, which leads to sepsis and bacteremia[5-7].

Like the same, the genus Staphylococci can be considered as normal flora on the skin but becomes opportunistic among patients receiving long term antimicrobial treatment[8]. Other members like *Streptococcus pneumonia*, *Salmonella* species, *E. coli*, *K. pneumoniae*, *Proteus mirabilis,* and *Mycobacterium tuberculosis* are emerging as important secondary infector in immune-compromised patients[9,10]. The main concern in these cases is the antibiotic resistance exhibited by these opportunistic pathogens. Because of constant exposure towards antibiotics and in close contact with pathogens, these opportunistic pathogens can develop resistance through gene transfer or by mutation[11,12].

Antimicrobial-resistant (AMR) can be addressed in various levels like multidrug-resistant (MDR), extensive drug-resistant (XDR), totaldrug-resistant (TDR) and pan drug-resistant (PDR) which are called as superbugs[13-17]. The ESKAPE, an acronym for the important causatives of nosocomial infections *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species and other opportunistic pathogens like *Escherichia coli, Shigella* species and *Proteus mirabilis*, is the main source of life-threatening nosocomial infections and are with increased levels of AMR[18-20].

The strategy behind antibiotic resistance advancement is a worldwide concern for health sector[21-23]. The bacterial strains are enduring to become more resistant to recent drugs, and the antibiotic pipeline is still diminishing, and this difficult situation continues unaware by the majority of the public which has led to the current situation where annually about more than 13 million people worldwide are dead due to infectious diseases alone[24]. Approximately 2 million infections and 23,000 fatalities in the United States are triggered annually by pathogenic antibiotic resistance. In Europe, 25,000 individuals suffer annually from bacteria susceptible to antibiotics[25]. Bacteria are discovered to be resistant to antibiotics owing to the broad accessibility and inappropriate use and dispose of antibiotics. India was the world's major consumer of antibiotics for human health in 2010 as 12.9 x 10^9 units (10.7 units per person). The second major consumers were China at 10.0 x 10^9 units (7.5 units per person) followed by the US at 6.8 x 10^9 units (22.0 units per person)[24]. It is anticipated that 300 million individuals will die early from drug resistance over the next 35 years[26]. As per the global impact of antimicrobial resistance research and interventions, most countries reduce unnecessary usage of antimicrobials[27].

To overcome the problem of antibiotic resistance, numerous methods are being experimented, and a number of drug classes are being analysed regularly for managing and controlling pathogenesis. Antibiotics from natural sources like plants are regarded to be effective in controlling pathogens[28]. Piperidines plays significant role in the synthesis of numerous pharmaceuticals. Piperidine, a heterocyclic moiety consists of six-membered rings which comprise of five methylene groups (-CH_2-) and one amine group (-NH-). Piperidines are naturally found in black pepper (*Piper nigrum*) and barley. The piperidine skeleton containing species are significant in the synthesis of organic compounds[29], including pharmaceuticals[30]. In recent days, piperidine scaffolds have been exploited into preclinical and clinical testing. Piperidines are found to exhibit wide range of biological properties viz., antibacterial, anticonvulsant, antihypertensive, anti-inflammatory, and antimalarial activity[31].

This study has been conducted to analyse the prevalence of bacterial and opportunistic

pathogens in various parts of TamilNadu, India and also to analyse their susceptibility pattern to various antimicrobial that is prescribed routinely and also against few isolated piperidine compounds.

MATERIALS AND METHODS

This research study was conducted to isolate multi-drug resistant opportunistic pathogen. Samples from different multi-specialty hospitals were collected in and around North-Western parts of Tamil Nadu, India.

Sample processing

Total of 238 clinical samples such as aspirates and pus samples of abscesses, surgical, accidental wound infections, and urinary catheter along with urine samples were collected from both inpatients and outpatients of multi-speciality hospitals. Samples were collected from surgical wound patients after three days of surgery in the hospital with the help of staff nurse by obtaining prior permission from the hospital and patient. The samples were collected aseptically and immediately transferred to the microbiology laboratory for further study.

Identification of isolates

A swab of aspirates, pus and catheters was serially diluted and inoculated in Luria Bertani (LB) broth and incubated at 37°C. After incubation for about 24 h, one loopful of the culture was streaked on selective agar medium. The selective agar medium that was used for the isolation of enteric pathogens is tabulated (Table 1). Further, the isolates were identified by Gram's staining and series of biochemical tests.

Antimicrobial susceptibility testing

For susceptibility screening, the Kirby-Bauer disc-diffusion technique on Mueller-Hinton agar (MHA) plates were used. As proposed by the Clinical and Laboratory Standards Institute (CLSI), antibiotic disc strengths were used. Susceptibility and resistance testing criteria of CLSI were followed[32]. Nearly ten antibiotics were used to study the isolates' antimicrobial sensitivity pattern and the antibiotics found to be prescribed to patients on a routine basis were used. Inhibition zones were evaluated around the antibiotic disks in the plates using a standard measuring scale, evaluating their amount of sensitivity

Quantitative antibacterial activity assay of piperidine compounds

Minimum inhibitory concentration (MIC) of piperidine compounds against the isolated pathogens was determined by measuring the OD value at 600 nm. DMSO was used as a solvent blank. PM3DMP (3,3-dimethyl-2,6-diphenyllpiperidine), TSPM3DMPO (1-toluenesulfonyl-3,3-dimethyl-2,6-iphenyllpiperidin-4-one), BPM3DMPO (1-Benzoyl-3,3-dimethyl-2,6-diphenyllpiperidin-4-one), BSPM3DMPO (1-Benzenesulfonyl-3,3-dimethyl-2,6-diphenyllpiperidin-4-one) and MCPM3DMPO (Impure and wrong compound not confirmed by NMR) are the piperidine compounds used in this study. DMSO and piperidine compounds in the varying concentration ranging from 6.25, 12.5, 25, 50, 100, and 200 µg/ml were added in 96-well microtitre plate. Bacterial cultures grown overnight were adjusted to 0.5 McFarland standard, and from that 100µL were added to each well. The Positive control titer well was added with sterile broth without any test compound. Microdilution plates sealed with a tight lid before incubation to prevent desiccation and

Table 1. Selective medium for isolation of pathogens

Selective media	Bacterial genera
Mannitol Salt Agar	*Staphylococcus sp.*
Xylose Lysine Deoxycholate Agar	*Salmonella sp.*
Eosin Methylene Blue agar (EMB)	*E. coli*
Mac Conkey Agar	*Klebsiella* sp.
Nutrient Agar (NA)	*Proteus* sp.
King's B medium	*Pseudomonas* sp.

Table 2. Demographic data of clinical specimens

No.	Types of sample	No. of sample Collected	No. of positive sample	No. of negative sample
1.	Pus	63	31	32
2.	Pus Aspirate	47	07	40
3.	Urine	71	14	57
4.	Urinary Catheter	13	04	09
5.	Abscesses	44	09	35
	Total	238	65	173

Table 3. Typical biochemical profile of Isolates

Gram's staining	Indole	MR	VP	Urease	TSI	Catalase	Glucose	Lactose	Maltose	Sucrose	motility	Oxidase	Suspected organism
G+ve Cocci	-	+	+	+	+	+	+	+	+	+	-	-	*Staphylococcus* sp.
G-ve Rods	-	+	-	-	+	+	+	-	+	-	+	-	*Salmonella* sp.
G-ve Rods	-	-	-	-	+	+	+	-	-	-	+	+	*Pseudomonas* sp.
G-ve Rod	-	+	-	+	+	+	+	-	-	+	+	-	*Proteus* sp.
G-ve Rod	-	-	+	-	+	+	-	+	-	-	+	-	*E. coli*
G-ve Rod	-	+	-	+	+	+	+	+	+	+	-	-	*Klebsiella* sp.

(+) Positive; (-) Negative

contamination. Incubate the plates at 37°C for 24 h and MIC was determined.

RESULTS

Isolation and identification of pathogens

About 167 swab samples from wounds, pus, abscesses, urinary catheters, and 71 urine samples were collected aseptically and transferred in the microbiology laboratory for further processing. The samples were serially diluted and inoculated in LB broth. After incubation, based on colony morphology, a single colony was selected, and loop full of culture was streaked on Nutrient agar for isolation of pure colony (Table 2). The pure colony obtained was subjected to Gram staining and biochemical screening. The results obtained were tabulated in Table 3. After that, the isolates were streaked on to selective medium for further confirmation. The organism and the sourced from which it was isolated were tabulated in Table 4.

Antibiogram of the isolates

The antibiogram study was performed on the positive isolates, where 17 isolates that showed consistent growth were selected and screened for the antibiotic sensitivity against 10 commercially available antibiotics. About 100% of isolates showed resistance towards Ampicillin and Chloramphenicol, 94% of resistance was noticed against Amikacin and Ciprofloxacin, 88% against Amoxicilin and Erythromycin, 82% against Cefazolin, 73% towards Azithromycin and 53% against cefdinir respectively. The percentage of resistance was notices against Carbenicillin (41%). *E. coli* isolates exhibited the highest degree

Table 4. Identification of Isolates

No.	Isolate	No. of Positives	Source
1.	*Staphylococcus* sp.	16	Pus (9), Urine (2), Abscesses (5)
2.	*Salmonella* sp.	03	Abscesses (3)
3.	*Pseudomonas* sp.	06	Pus Aspirate (5), Urine (1)
4.	*Proteus* sp.	04	Pus (3), Urine (1)
5.	*E. coli*	11	Pus (2), Urine (7), Pus Aspirate (1), Catheter (1)
6.	*Klebsiella* sp.	07	Pus (4), Urine (3S)

of resistance among the isolated pathogens (Table 5).

The minimum inhibitory concentration of piperidine compounds

One isolate from each organism, which showed the highest drug resistance was selected for studying the MIC of piperidine compounds. DMSO was used as blank. About 100µL of predetermined culture was added to all the titer well. The absorbance value of *S. aureus* was noticed as 0.72. Piperidine compound at the concentration of 200µg/mL showed complete inhibition of the test isolates (Table 6). Whereas, the absorbance of *Salmonella* sp. obtained was 0.99. PM3DMP showed the highest inhibition at the concentration on 12.5µg/mL, followed by 25µg/mL concentration of MCPM3DMPO. The least inhibition was noticed in BSPM3DMPO (Table 7). MCPM3DMPO showed complete inhibition against *Pseudomonas* sp. at the concentration of 12.5µg/mL. The least inhibition was noticed in TSPM3DMPO (Table 8). The positive control exhibited the OD valve 1.10, respectively.

MCPM3DMPO and TSPM3DMPO showed complete inhibition of test isolate at 100µg/mL concentration against the density of *Proteus* sp. registered as 1.02 (Table 9). As like *Staphylococcus* sp., *Proteus* sp. also showed the least susceptibility towards test isolates. *E. coli* showed the highest resistance towards piperidine compounds as they exhibited against antibiotics. Among the test compound, MCPM3DMPO alone inhibited the pathogen at 50µg/mL (Table 10). *Klebsiella* sp. showed the least resistance towards tested

Table 5. Antibiotic Resistance of Isolates

No	Isolate	Organism	Ak	Ac	Am	Az	Cb	Cz	Ci	C	Cd	E
1.	CVST1	*Staphylococcus sp.*	26	24	23	16	0	28	30	31	38	37
2.	CVST2	*Staphylococcus sp.*	25	22	27	15	0	30	35	32	33	35
3.	CVST3	*Staphylococcus sp.*	26	27	24	14	0	32	31	34	38	37
4.	CVST4	*Staphylococcus sp.*	19	20	17	9	0	13	13	31	18	34
5.	CVST5	*Staphylococcus sp.*	24	24	26	14	0	21	16	30	33	36
6.	CVSA1	*Salmonella sp.*	24	26	21	14	0	15	34	17	0	18
7.	CVPS1	*Pseudononas sp.*	25	30	18	0	0	0	22	15	0	18
8.	CVPS2	*Pseudononas sp.*	28	27	13	0	25	0	25	22	0	18
9.	CVPR1	*Proteus sp.*	12	23	23	22	16	22	28	26	0	10
10.	CVPR2	*Proteus sp.*	18	25	24	22	15	20	30	30	0	0
11.	CVEC1	*E. coli*	26	21	26	0	24	20	17	30	7	12
12.	CVEC2	*E. coli*	24	20	23	15	28	29	15	28	10	18
13.	CVEC3	*E. coli*	27	21	25	14	30	26	18	28	9	15
14.	CVEC4	*E. coli*	0	0	19	0	0	12	16	27	0	16
15.	CVEC5	*E. coli*	12	0	24	0	0	24	17	36	0	16
16.	CVKL1	*Klebsiella sp.*	20	18	12	24	22	0	0	19	0	0
17.	CVKL2	*Klebsiella sp.*	32	20	16	0	0	20	20	30	8	14

Table 6. MIC of Piperidines against *Staphylococcus* sp. at 600 nm

No.	Test compounds	I dilution 200µg/mL	II dilution 100µg/mL	III dilution 50µg/mL	IV dilution 25µg/mL	V dilution 12.5µg/mL	VI dilution 6.25µg/mL	Culture control
1	PM3DMP	-0.07	0.01	0.07	0.09	0.12	0.13	
2	TSPM3DMPO	-0.03	0.04	0.09	0.11	0.16	0.19	
3	BPM3DMPO	-0.03	0.04	0.09	0.11	0.16	0.19	0.72
4	SPM3DMPO	-0.01	0.07	0.09	0.17	0.2	0.27	
5	CPM3DMPO	0	0.03	0.04	0.07	0.17	0.2	

compounds. TSPM3DMPO and MCPM3DMPO inhibited the growth of *Klebsiella* at 6.25 µg/mL concentration, respectively (Table 11).

DISCUSSION

In medical literature, opportunistic pathogens are typically characterized as pathogenic when they get favourable host conditions such as ageing, illness, injury, medication, immunodeficiency and previous infection. They emerge from the roots of normal commensal symbionts or microbes obtained from the environment. Acquiesce may be from a diseased person or hospital setting, which can be

Table 7. MIC of Piperidines against *Salmonella* sp. at 600 nm

No.	Test compounds	I dilution 200µg/mL	II dilution 100µg/mL	III dilution 50µg/mL	IV dilution 25µg/mL	V dilution 12.5µg/mL	VI dilution 6.25µg/mL	Culture control
1	PM3DMP	-0.02	-0.3	-0.32	-0.35	-0.38	0.02	0.99
2	TSPM3DMPO	0.04	0.19	0.27	0.31	0.31	0.32	
3	BPM3DMPO	0	0	0.09	0.22	0.32	0.39	
4	BSPM3DMPO	0.12	0.22	0.39	0.41	0.42	0.59	
5	MCPM3DMPO	0	-0.07	-0.09	-0.11	0.02	0.2	

Table 8. MIC of Piperidines against *Psuedomonas* sp. at 600 nm

No.	Test compounds	I dilution 200µg/mL	II dilution 100µg/mL	III dilution 50µg/mL	IV dilution 25µg/mL	V dilution 12.5µg/mL	VI dilution 6.25µg/mL	Culture control
1	PM3DMP	-0.09	-0.02	0	0.01	0.14	0.19	1.10
2	TSPM3DMPO	0	0.06	0.13	0.15	0.16	0.19	
3	BPM3DMPO	-0.04	-0.03	0	0.03	0.07	0.1	
4	BSPM3DMPO	-0.02	0.01	0.04	0.07	0.1	0.13	
5	MCPM3DMPO	-0.11	-0.09	-0.06	-0.02	0	0.09	

Table 9. MIC of Piperidines against *Proteus* sp. at 600 nm

No.	Test compounds	I dilution 200µg/mL	II dilution 100µg/mL	III dilution 50µg/mL	IV dilution 25µg/mL	V dilution 12.5µg/mL	VI dilution 6.25µg/mL	Culture control
1	PM3DMP	-0.02	0.01	0.05	0.07	0.14	0.16	1.02
2	TSPM3DMPO	-0.02	-0.02	0.01	0.09	0.14	0.17	
3	BPM3DMPO	-0.01	0.03	0.05	0.08	0.11	0.18	
4	BSPM3DMPO	0	0.02	0.02	0.04	0.07	0.31	
5	MCPM3DMPO	0	0	0.01	0.09	0.11	0.13	

Table 10. MIC of Piperidines against *E. coli a*t 600 nm

No.	Test compounds	I dilution 200µg/mL	II dilution 100µg/mL	III dilution 50µg/mL	IV dilution 25µg/mL	V dilution 12.5µg/mL	VI dilution 6.25µg/mL	Culture control
1	PM3DMP	0.04	0.04	0.08	0.15	0.18	0.18	0.96
2	TSPM3DMPO	0.05	0.07	0.09	0.09	0.14	0.14	
3	BPM3DMPO	0.02	0.04	0.06	0.07	0.08	0.16	
4	BSPM3DMPO	0.04	0.12	0.15	0.16	0.17	0.19	
5	MCPM3DMPO	-0.02	0	-0.04	0.02	0.02	0.09	

Table 11. MIC of Piperidines against *Klebsiella* sp. at 600 nm

No.	Test compounds	I dilution 200µg/mL	II dilution 100µg/mL	III dilution 50µg/mL	IV dilution 25µg/mL	V dilution 12.5µg/mL	VI dilution 6.25µg/mL	Culture control
1	PM3DMP	-0.02	-0.02	-0.01	0	0.01	0.05	
2	TSPM3DMPO	-0.06	-0.09	-0.12	-0.03	-0.02	-0.02	
3	BPM3DMPO	0.03	0.08	0.09	0.14	0.18	0.2	0.93
4	BSPM3DMPO	-0.02	0	0.01	0.03	0.05	0.08	
5	MCPM3DMPO	-0.04	-0.05	-0.08	-0.1	-0.11	-0.13	

highly pathogenic. Hence present study focused on opportunistic pathogens, its antimicrobial resistance property, and effectiveness of alternative therapeutic compounds.

About 167 swab samples from wounds, pus, abscesses, urinary catheters, and 71 urine samples were collected in this study for the isolation of pathogens. Among them, 47 reported positive for the presence of pathogens like *Staphylococcus* species (16), *Salmonella* species (3), *Pseudomonas* species (6), *Proteus* species (4), *E. coli* (11) and *Klebsiella* species (7). Similarly, Salmani *et al.*[33]. and Hoque *et al.*[34]. isolated *P. aeruginosa* from various clinical specimens. Upreti *et al.*[35]*.* also recorded high occurrence of MDR (68.2% of *S. aureus*, 80% of *E. coli*, 50% of *Proteus* sp., 80% of *P. aeruginosa* and 77.7% of CoNS), MRSA 60.6% (40/66) and ESBL (25% of *E. coli*, 40% of *K. pneumonia* and 33.3% of *C. freundii*) from pus samples.

In the present research, the most frequently obtained organism was *S. aureus* and *E. coli*. Similarly, Feleke *et al.*[36]*.* obtained 77 (35.6%) of *S. aureus*, followed by *E. coli* 33 (15.3%) and *Klebsiella* spp 29 (13.4%). Lilani *et al.*[37]*.* obtained 16 isolates from 14 infected wounds samples. *S. aureus* was the commonest isolate followed by Pseudomonas along with *E. coli* (2) and *Klebsiella* sp (1). This was additionally supported by Hussein[38] study, in which he isolated 22 *P. aeruginosa*, 18 *E. coli* and 15 *E. cloacae* and 18 *P. mirabilis*.

We consider that the antibiotic resistance was reported after the invention of Penicillin, but resistance to antibiotic variety have been discovered in ancient DNA from 30,000-year-old permafrost residues[39]. This antibiotic resistance study plays an significant role in determining better therapeutic compound. In the present study, the test isolates were screened for antibiotic resistance against 10 antibiotics.

Feleke *et al.*[36]. recorded varying degree of resistance *S. aureus E. coli, Klebsiella* species, *Citrobacter* species, *E. aerogenes.* The highest number of MDR isolates were documented in his study in *Citrobacter* species (100%), *Klebsiella* species (79.3%), *E. coli* (75.8%), and *S. aureus* (61%). In this research, the general MDR resistant durable was (70.4%), and about 94% of resistance was noticed against amikacin and ciprofloxacin, 88% against amoxicillin and erythromycin, 82% against cefazolin, 73% towards azithromycin and 53% against cefdinir respectively.

Similarly, Salmani *et al.*[33]*.* screened antibiotic resistance against *P. aeruginosa* using single and antibiotics in combination. The highest sensitivity was against the combination of drugs like piperacillin and tazobactam (93.5%). Highest resistance rate was seen for amoxicillin followed by doxycycline. Similarly, increased percentage of sensitivity were observed by Singh *et al.*[40]. In the research conducted by Hoque *et al.*[34]*. P. aeruginosa* showed higher resistance to penicillin (98.98%) followed by cephalosporins (89.85%). The combination of piperacillin and tazobactam (3.37%) was found to be most sensitive.

Pawar *et al.*[41]. isolated *Klebsiella* species (466), *Acinetobacter* species (377), *Escherichia coli* (368), and *Pseudomonas aeruginosa* (311) from various clinical sample and samples from patients in intensive care units as we isolated in the current study. Pawar *et al.*[41]. also studied antibiotic resistance of *E. coli*, *Klebsiella* species, *Acinetobacter* species and *P. aeruginosa* and obtained varying resistance pattern according to the isolates. Sohail *et al.*[42]*.* observed *E. coli* as extremely antimicrobial resistant, viz.

cephalexin (95%), cephradine (95%), pipemidic acid (92%), amikacin (91%), and nalidixic acid (91%). ג-lactam antibiotics like aztreonam, ampicillin, and amoxicillin/clavulanic acid, that were routine were also futile against *E. coli*. Similarly, Deshmukh *et al.*[43]. conclude their finding as *E. coli* isolates were highly resistant towards all antibiotic used in his study. This supports the results of the present study that *E. coli* isolates exhibited the highest degree of resistance among the isolated pathogens.

Antibiotic resistance of the pathogens made us focus on an alternative like including phytochemicals, probiotics, antimicrobial peptide, bacteriophages, and phage lytic enzymes were assessed to develop therapies to manage systemic/ invasive rather than superficial infections which also been as the main present lines of the research area[44]. These alternatives can assist us in alleviating the problem of resistance in two respects. First, they can be used for infection management and as a substitute for antibiotics[45]. In this context, there is evidence that Piperidine compounds are also expressed the promising results as alternative therapeutics against this pathogen. In the present study, one isolate from each organism, which shows the highest drug resistance was selected for studying the MIC of piperidine compounds.

The compounds were diluted using DMSO and to obtain a concentration of 6.25 to 200 µg/mL, and microbial growth was evaluated by absorbance readings (Abs) at 600 nm. Similarly, Han *et al.*[46]*.* studied MIC of hydrazide-hydrazones derived from Benzocaine, and he noticed MIC was defined at the lowest concentration of the compounds to inhibit the growth of microorganisms and his results were supported by Kalaycı *et al.*[47]. As the same in the present study, all test compounds showed inhibition at the lowest concentration. As like *Staphylococcus* species and *Proteus* species also showed the least susceptibility towards test isolates. *E. coli* showed the highest resistance towards Piperidine compounds as they exhibited against antibiotics. *Klebsiella* species. showed the least resistance towards tested compounds.

Imran *et al.*[48]*.* synthesized 2-piperidino derivatives compounds and screened for antimicrobial activity in diffusion method. One of his compound - 6a (MIC = 50µg/mL) exhibited the increased activity against *S. aureus*, *E. faecalis*, *S. epidermidis, B. subtilis* and *B. cereus.* But in the case of the present study, Piperidine compound at the concentration 200µg/ml showed complete inhibition of the *S. aureus*. Similarly, Compound 6a had further exhibited good activity (MIC = 25µg/mL) against *E. coli*, *K. pneumonia, P. aeruginosa, P. vulgaris* and *B. bronchiseptica.* His results were supported by *Imran et al.*[48]. Similarly, PM3DMP showed the highest inhibition at the concentration on 12.5µg/mL, followed by 25µg/mL concentration of MCPM3DMPO against *Salmonella* species. MCPM3DMPO showed complete inhibition against *Pseudomonas* species at 12.5µg/mL concentration. Among the test compound, MCPM3DMPO alone inhibited *E. coli* at 50µg/mL. TSPM3DMPO and MCPM3DMPO inhibited the growth of *Klebsiella* at 6.25µg/mL concentration, respectively. But MCPM3DMPO and TSPM3DMPO showed complete inhibition of test isolate at 100µg/mL concentration against *Proteus* species. These results are supported by one another study of Imran *et al.*[49]*.* Desai *et al.*[50]. synthesised compound which exhibited excellent activity against *E. coli* at MIC 50µg/mL, *P. aeruginosa* at MIC 100µg/mL, *S. aureus* at MIC 100µg/mL and 50µg/mL respectively.

Duruskari *et al.*[51]*.* used well diffusion methods for screening potential inhibition activity of synthesised piperidine compounds. At 0.1 %, the test compound showed the better result when tested with the *A. baumannii* and *P. aeruginosa*, while at the concentration of 0.05 % inhibition zones were similar for all studied pathogens except *K. pneumoniae*. But inhibition was not detected against *E. coli* and exhibited the lowest activity when tested with *S. aureus.* Kumar and Joshi[52] tested their freshly synthesized diazepine compounds and screened for antibacterial activity against *K. pneumoniae* and *S. aureus* and also other bacterial species by using well diffusion method. The findings show that these compounds were effective against all the tested organisms.

CONCLUSION

Hence from this preliminary *in vitro* study, we conclude that the synthesized piperidine compound exhibited a remarkable antimicrobial potency towards isolated opportunistic pathogens. All compounds exhibited potential inhibition activity against both Gram-positive and negative

bacteria. These results give us insight about the efficiency of alternative therapeutics compounds against the pathogens and need for development for new compounds to overcome antibiotic resistance in pathogens. Thus, it can, therefore, be regarded as a successful lead in the further growth and design of new chemical entities. Therefore, our research conclusion will provide a significant impact on further investigations of other piperidine derivatives in search of new molecules having potent antimicrobial activity.

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None.

CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

AUTHORS' CONTRIBUTION

RV and TV have equally contributed to this study in designing the study, carrying out laboratory works, collected and analysed the data, and also prepared the manuscript.

DATA AVAILABILITY

All data generated or analysed during this study are included in this published article. If any specific data required, then available on request from the authors.

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ETHICS STATEMENT

All text, data, figures/tables or other illustrations presented in the manuscript are completely original and does not contain or include material taken from other copyrighted sources. This article does not contain any studies about human or animal objects.

REFERENCES

1. Winkelhoff AJ, Rams TE, Slots J Systemic antibiotic therapy in periodontics. *Periodontol*, 2000; **10**: 45-78. https://doi.org/10.1111/j.1600-0757.1996.tb00068.x.
2. Flynn MJ, Slots J. Beta-hemolytic streptocolcci in advanced periodontitis. *Oral Microbiol. Immunol.*, 1993; **8**: 295-297. https://doi.org/10.1111/j.1399-302X.1993.tb00577.x.
3. Slots J, Rams TE, Feik D, Taveras HD, Gillespie GM. Subgingival microflora of advanced periodontitis in the Dominican Republic. *J. Periodontol.*, 1991; **62**: 543-547. https://doi.org/10.1902/jop.1991.62.9.543.
4. Querido SM, Back-Brito GN, Dos Santos SS, Leто MV, Koga-Ito CY, Jorge AO. Opportunistic microorganisms in patients undergoing antibiotic therapy for pulmonary tuberculosis. *Braz. J. Microbiol.*, 2011; **42**: 1321–1328. https://doi.org/10.1590/S1517-83822011000400012.
5. Kaklamanos EG, Charalampidou M, Menexes G, Topitsoglou V, Kalfas S. Transient oral microflora in Greeks attending day centres for the elderly and residents in homes for the elderly. *Gerodontology*, 2005; **22**: 158-167. https://doi.org/10.1111/j.1741-2358.2005.00069.x.
6. Velge P, Cloeckaert A, Barrow P. Emergence of Salmonella epidemics: the problem related to Salmonella enterica serotype enteritidis and multiple antibiotic resistance in other major serotypes. *Vet. Res.*, 2005; **36**: 267-288. https://doi.org/10.1051/vetres:2005005.
7. Abraham M, De N, Sudi IY, Maori L. Isolation of methicillin resistant *Staphylococcus aureus* (MRSA) from AIDS patients attending the state specialist hospital, Yola and Federal Medical Centre, Yola, Adamawa State, Nigeria. *Report and Opinion*, 2009; **1**: 103-107.
8. Agarwal R, Gupta D, Ray P, Agarwal A, Jindal SK. Epidemiology, risk factors and outcome of nosocomial infections in a respiratory intensive care unit in North India. *J. Infect.*, 2006; **53**: 98-105. https://doi.org/10.1016/j.jinf.2005.10.021.
9. Jaspan HB, Huang LC, Cottn MF, Whitelaw A, Myer L. Bacterial disease and antimicrobial susceptibility pattrns in HIV-infected, hospitalized children: A retrospective cohort study. *PLoS One*, 2008; **3**: 16. https://doi.org/10.1371/journal.pone.0003260.
10. Santajit S, Indrawattana N. Mechanisms of antimicrobial resistance in ESKAPE pathogens. *Biomed. Res. Int.*, 2016; **10**: 2475067. https://doi.org/10.1155/2016/2475067.
11. Kalluru S, Eggers S, Barker A, Shirley D, Sethi AK, Sengupta S, Yeptho K, Safdar N. Risk factors for infection with multidrug-resistant organisms in Haryana, India. *Am. J. Infect. Control*, 2017; **46**: 341-345. https://doi.org/10.1016/j.ajic.2017.08.021.
12. Chandra H, Bishnoi P, Yadav A, Patni B, Mishra AP, Nautiyal AR. Antimicrobial resistance and the alternative resources with special emphasis on plant-based antimicrobials- a review. Plants, 2017; **6**: 16. https://doi.org/10.3390/plants6020016.
13. Levy SB. Factors impacting on the problem of antibiotic resistance. *J. Antimicrob. Chemother.*, 2002; **49**: 25-30. https://doi.org/10.1093/jac/49.1.25.
14. Alekshun MN, Levy SB. Molecular mechanisms of antibacterial multidrug resistance. *Cell*, 2007; **128**: 1037-1050. https://doi.org/10.1016/j.cell.2007.03.004.
15. Lee DR, Cheng J, Lee SK, Hong HJ, Song J, Yang SH, Suh JW. Antimulti drug resistant pathogen activity of siderochelin A, produced by a novel Amycolatopsis sp. KCTC 29142. *Kor. J. Microbiol.*, 2016; **52**: 327-335. https://doi.org/10.7845/kjm.2016.6033.

16. Exner M, Bhattacharya S, Christiansen B, Gebel J, Goroncy-Bermes P, Hartemann P. Antibiotic resistance: what is so special about multidrug-resistant Gram-negative bacteria?. *GMS Hyg. Infect. Control*, 2017; **12**: 05.
17. Spengler G, Kincses A, Gajdacs M, Amaral L. New roads leading to old destinations: efflux pumps as targets to reverse multidrug resistance in bacteria. *Molecules*, 2017; **22**: 468. https://doi.org/10.3390/molecules22030468.
18. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. *Perspect. Medicin Chem.*, 2014; **6**: 25-64. https://doi.org/10.4137/PMC.S14459.
19. Prasch S, Bucar F. Plant derived inhibitors of bacterial efflux pumps: an update. *Phytochem. Rev.*, 2015; **14**: 961. https://doi.org/10.1007/s11101-015-9436-y.
20. Cerceo E, Deitelzweig SB, Sherman BM, Amin AN. Multidrug resistant gram-negative bacterial infections in the hospital setting: overview, implications for clinical practice, and emerging treatment options. *Microb. Drug Resist.*, 2016; **22**: 412-431. https://doi.org/10.1089/mdr.2015.0220.
21. Perveen I, Majid A, Knawal S, Naz I, Sehar S, Ahmed S, Raza MA. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* and Coagulase-Negative Staphylococci in Rawalpindi, Pakistan. *British Journal of Medicine and Medical Research*, 2013; **3**: 198-209. https://doi.org/10.9734/BJMMR/2013/2109.
22. Chellat MF, Raguz L, Riedl R. Targeting antibiotic resistance. *Angew Chem. Int. Ed. Engl.*, 2016; **15**: 6600-6626. https://doi.org/10.1002/anie.201506818.
23. Schindler BD, Kaatz GW. Multidrug efflux pumps of Gram-positive bacteria. *Drug Resist Updat*, 2016; **27**: 1-13. https://doi.org/10.1016/j.drup.2016.04.003.
24. Gislin D, Sudarsanam D, Raj GA, Baskar K. Antibacterial activity of soil bacteria isolated from Kochi, India and their molecular identification. *J. Genetic Engineering and Biotechnol.*, 2018; **16**: 287-294. https://doi.org/10.1016/j.jgeb.2018.05.010.
25. Humphries RM, Linscott AJ. Laboratory diagnosis of bacterial gastroenteritis. *Clin. Microbiol. Rev.*, 2015; **28**: 3-31. https://doi.org/10.1128/CMR.00073-14.
26. O'Neill J. Tackling drug-resistant infections globally: Final report and recommendations. The review on antimicrobial resistance; London: HM Government and the Wellcome Trust. 2016.
27. Das B, Chaudhuri S, Srivastava R, Nair GB, Ramamurthy T. Fostering research into antimicrobial resistance in India. *BMJ*, 2017; **358**: 3535. https://doi.org/10.1136/bmj.j3535.
28. Bhakyashree K, Kannabiran K Actinomycetes mediated targeting of drug resistant MRSA pathogens. *Journal of King Saud University*, 2018 (In Press, Corrected Proof). https://doi.org/10.1016/j.jksus.2018.04.034.
29. Takahata H, Banba Y, Ouchi H, Nemoto H, Kato A, Adachi I. Asymmetric synthesis of the four possible fagomine isomers. *J. Org. Chem.*, 2003; **68**: 3603-3607. https://doi.org/10.1021/jo034137u.
30. Misra VS, Singh S, Agarwal R, Chaudhary KC. Synthesis and antimicrobial activity of new substituted 1,3,5-triazine derivatives. *J. Chem. Soc. Pak.*, 1981; **3**: 209.
31. Brahmachari G, Choo C, Ambure P, Roy K. In vitro evaluation and in silico Screening of synthetic acetylcholinesterase inhibitors bearing functionalized piperidine pharmacophores. *Bioorganic Med. Chem.*, 2017; **23**: 4567-4575. https://doi.org/10.1016/j.bmc.2015.06.005.
32. Clinical and Laboratory Standards Institute., Performance standards for antimicrobial susceptibility testing: eighteenth informational supplement: approved standards M100-S18. Clinical and Laboratory Standards Institute, Wayne, PA; 2008.
33. Salmani MP, Mindolli PB. Isolation, Identification and antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* from clinical isolates in a tertiary care centre. *Int. J. Curr. Microbiol. App. Sci.*, 2015; **4**: 968-972.
34. Hoque MM, Ahmad M, Khisa S, Uddin MN, Jesmine R. Antibiotic resistance pattern in *Pseudomonas aeruginosa* isolated from different clinical specimens. *KYAMC Bangladesh*, 2015; **11**. https://doi.org/10.3329/jafmc.v11i1.30669.
35. Upreti N, Rayamajhee B, Sherchan SP, Choudhari MK, Banjara MR. Prevalence of methicillin resistant Staphylococcus aureus, multidrug resistant and extended spectrum ג-lactamase producing gram negative bacilli causing wound infections at a tertiary care hospital of Nepal. *Antimicrob. Resist. Infect. Control*, 2018; **7**: 121. https://doi.org/10.1186/s13756-018-0408-z.
36. Feleke T, Eshetie S, Dagnew M, Endris M, Abebe W, Tiruneh M, Moges F. Multidrug-resistant bacterial isolates from patients suspected of nosocomial infections at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia. *BMC Res Notes*, 2018; **11**: 602. https://doi.org/10.1186/s13104-018-3709-7.
37. Lilani SP, Jangale N, Chowdhary A, Daver GB. Surgical site infection in clean and clean contaminated cases. *Indian Journal of Medical Microbiology*, 2005; **23**: 249-252.
38. Hussein EF. Isolation, Identification and antibiotic susceptibility of pathogenic bacteria isolated from clinical samples. *IOSR Journal of Pharmacy and Biological Sciences*, 2016; **11**: 27-39. https://doi.org/10.9790/3008-1104022739.
39. D'Costa VM, King CE, Kalan L, Morar M, Sung WW, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB, Poinar HN, Wright GD. Antibiotic resistance is ancient. *Nature*, 2011; **477**: 457-461. https://doi.org/10.1038/nature10388.
40. Singh AA, Kaur M, Singh A, Goel S, Surana A, Bhardwaj A. Prevalence of microbial infection and strategic pattern of antimicrobial resistance among intensive care unit patients in a tertiary care teaching hospital from rural Northern India. *Int. Arch. Integr. Med.*, 2015; **2**: 14-20. https://doi.org/10.5812/jjm.19272v2.
41. Pawar SK, Patil SR, Karande GS, Mohite ST, Pawar VS. Antimicrobial sensitivity pattern of clinical isolates in intensive care unit in a tertiary care hospital from Western India. *Int. J. of Scientific Study*, 2015; **4**: 263.
42. Sohail M, Khurshid M, Saleem HG, Javed H, Khan AA.

Characteristics and antibiotic resistance of urinary tract pathogens isolated from Punjab, Pakistan. *Jundishapur J. Microbiol.*, 2015; **8**: 19272.

43. Deshmukh PM, Ukesh CS, Patil SD. Antibiotic resistance pattern of some pathogens isolated from clinical samples. Bulletin of Environment, *Pharmacology and Life Sciences*, 2016; **5**: 66-72.
44. Waldetoft WK, Brown SP. Alternative therapeutics for self-limiting infections- An indirect approach to the antibiotic resistance challenge. *PLoS Biol*, 2017; **15**: 2003533. https://doi.org/10.1371/journal.pbio.2003533.
45. Czaplewski L, Bax R, Clokie M, Dawson M, Fairhead H, Fischetti VA. Alternatives to antibiotics-A pipeline portfolio review. *Lancet Infect. Dis.*, 2016; **16**: 239-251. https://doi.org/10.1016/S1473-3099(15)00466-1.
46. Han M , G rol G, Yildirim T, Kalayci S, Sahin F, K n kg zel SG. Synthesis and antibacterial activity of new hydrazide-hydrazones derived from Benzocaine. *Marmara Pharmaceutical Journal*, 2017; **21**: 961-966. https://doi.org/10.12991/mpj.2017.34.
47. Kalayci S, Demirci S, Sahin F. Antimicrobial properties of various psychotropic drugs against broad range microorganisms. *Curr. Psychopharmacol.*, 2014; **3**: 195-202. https://doi.org/10.2174/22115560046661 50520230121.
48. Imran M, Abida, Alsalman AJ. Synthesis and evaluation of antimicrobial activity of some 2-morpholinomethylamino-4(7-unsubstituted/ substitutedcoumarin-3yl)-6-chlorosubstituted phenyl Pyrimidines. *Trop. J. Pharm. Res.*, 2016a; **15**: 393-404. https://doi.org/10.4314/tjpr.v15i2.24.
49. Imran M, Alam O and Abida 2016b, Synthesis and antimicrobial activity of some 2-Piperidinomethylamino-4- (7-H/substituted coumarin-3-yl) 6-chlorosubstituted phenyl pyrimidines. *Trop. J. Pharm. Res.*, 2016b, **15**: 1955-1965. https://doi.org/10.4314/tjpr.v15i9.21.
50. Desai NC, Makwana AH, Senta RD. Synthesis, characterization and antimicrobial activity of some new 4-(4-(2-isonicotinoyl hydraziny l)-6-((aryl) amino)-1,3,5-triazin-2-ylamino)- N-(pyrimidin-2-yl) benzenesulfonamides. *Journal of Saudi Chemical Society*, 2017; **21**: 25-34. https://doi.org/10.1016/j.jscs.2013.09.006.
51. Duruskar GSH, Maharramov MN, Hasanova USH, Ganbarov KHG, Eyvazova GM, Israyilova AA, Maharramov AM. Synthesis and Antimicrobial Properties of New Derivatives of Morpholine and Piperidine Based on 1-Chloro-3-methoxy-propylbenzene. *Asian Journal of Chemistry*, 2018; **30**: 269-272. https://doi.org/10.14233/ajchem.2018.20876.
52. Kumar R, Joshi YC. Synthesis and antimicrobial, antifungal and anthelmintic activities of 3 H-1, 5-benzodiazepine derivatives. *J. Serbian Chem. Soc.*, 2008; **73**: 937-943. https://doi.org/10.2298/JSC0810937K.