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Investigating novel compounds to combat Multidrug-Resistant bacterial infections

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Abstract

A major public health problem nowadays is the evolution of antimicrobial medication resistance on a global scale. Decades of study have not eliminated the devastating impact of bacterial illnesses on human health. These include TB, septicaemia, staphylococcal skin infections, pneumonia, and others. Microbes resistant to many regularly used antibiotics have emerged as a result of the overuse and misuse of antibiotics in recent decades, which might make future infections very challenging to cure. Due to the widespread medication resistance and, in particular, the appearance of their multidrug-resistant phenotypes, the management of these illnesses continues to be a significant issue in modern times. This highlights the critical need for new classes of antimicrobials that can effectively combat multidrug-resistant bacteria. Our findings provide light on the eternal possibilities of bacteria in this area, which is quite encouraging. The latest research confirms that uncommon actinomycetes might be a great source of new antimicrobial agents that effectively inhibit the development of many multidrug-resistant bacteria. Our research leads us to believe that uncommon actinomycetes and the bioactive metabolites they produce might be a viable alternative to conventional antibiotics in the fight against multidrug-resistant bacteria. Research into the use of microbial natural products in the pharmaceutical industry has a promising future.

Keywords: Antimicrobial susceptibility, bioactive extracts, multi-drug resistance, rare actinomycetes, S. *pneumoniae*, time-kill kinetics

Introduction

The alarming rise of bacteria and other microbes that are resistant to antibiotics is a growing problem in public health across the world. Alternative medications for the treatment of sensitive and multidrug-resistant bacterial infections are urgently required. The pursuit of novel, efficient Research initiatives throughout the globe continue to prioritise the development of antimicrobial medicines derived from natural sources. In order to control this issue, new antibiotics are urgently needed. As the number of harmful microbes that are resistant to antibiotics continues to rise, there is an urgent need for new antimicrobial medicines. It seems that the 'ailing pharmaceutical sector' is still not following the 'prescription of Demain,' as stated in 2002, as seen by the steadily declining antibiotic pipeline. Natural product discovery, high-throughput screening, genomics, proteomics, and combinatorial chemistry are all pillars of the drug industry's future success, as the author made plain; removing natural products from this mix would lead to disaster.

As of right now, the most encouraging source for novel antibiotics is still natural compounds (Tiwari and Gupta 2012) [5]. One of the most important ways to accomplish this enormous undertaking has long been bioprospecting. Such structural intricacy, variation, and uniqueness have likely never been seen in any other field of drug development. Multiple sources have argued that natural compounds, particularly those with a microbial origin, had an important role in the development of antibiotics (Demain 1998; Demain and Adrio 2008) [6, 7]. However, bacteria have played a pivotal role in the development of bioactive metabolites and antimicrobial medicines that have advanced medical care. Approximately sixty to eighty thousand metabolites, or naturally occurring chemicals, produced from bacteria have been reported (Berdy 2012) [8]. Approximately 33,000 molecules, or almost half of all microbial metabolites, display a biological activity, such as antibiotic or 'other' effects; traditional antibiotics make up around 40% (or 28,000 metabolites), whereas chemicals originating from plants account for 7% and those from

animals for 3% (Berdy 2012) [8]. Given the revelation When streptomycin was discovered in 1943, the actinomycetes, which are bacteria in the order Actinomycetales, came to the forefront of antibiotic drug research. According to Tiwari and Gupta (2012) [5] and Tiwari and Gupta (2014) [9], actinomycetes are without a doubt the most abundant source of naturally occurring bioactive chemicals in the modern world. It has been very productive to conduct systematic studies of natural compounds, particularly those produced from actinomycetes. According to Bordy (2005) [10], actinomycetes are responsible for the production of 45 percent of all bioactive natural compounds, including nearly half of all microbial antibiotics that have been found so far. More than 90% of antibiotics used in clinical practice come from actinomycetes, and these microbes also make up almost 2/3 of all bioactive compounds found in nature (Hamaki *et al*. 2005) [11]. In this setting, actinomycetes play a key role as microorganisms.

Literature Review

Ashwath, Priyanka *et al*. (2021) [1]. There is a growing public health and economic cost due to the fact that disease-causing microorganisms are becoming more resistant to antimicrobial treatments. Conventional antibiotics have become less effective in treating and preventing infectious illnesses due to the rise of multi-drug-resistant bacteria. Infections produced by drug-resistant bacteria are presently difficult to avoid, and there aren't many effective therapy regimens available. Bacteriophages, vaccinations, anti-biofilm peptides, and antimicrobial peptides are some of the alternative tactics that have been used recently to reduce and control infections' resistance to antibiotics. Nevertheless, this review delves into the innovative and strong molecular strategy of antisense RNA (asRNA) technology and CRISPR-based antibiotic therapy. These methods have the potential to eradicate drug-resistant bacteria in a sequence-specific way, opening up new possibilities for treating infections associated with multiple drug resistance.

Xu, Ze-Qi *et al*. (2013) [2]. One of the most pressing global health concerns is the rise of multidrug-resistant (MDR) bacterial infections, particularly those caused by Gram-negative pathogens. However, throughout the last fifty years, there has been little progress in developing new antibiotics to treat Gram-negative bacteria that have developed resistance. Research and development efforts to discover new antibiotics that can combat multidrug-resistant Gram-negative bacteria are summarised in this article. It highlights three important topics. The article begins by examining new compounds that mimic known antibiotics like β-lactams, tetracyclines, and aminoglycosides. It then moves on to discuss drugs that target new bacterial enzymes such aminoacyl-tRNA synthetase and peptide deformylase. Second, it looks at non-traditional methods, such as therapeutic antibodies, efflux pump inhibitors, siderophores, cationic antimicrobial peptides, and a resurgence of interest in withdrawn or underutilised therapies. Thirdly, the writers want to provide a rundown of where each medication candidate is in terms of clinical development. When faced with the enormous challenge posed by MDR superbugs, the conventional analogue method falls short. Considering the genomics approach's failure to provide potential new targets and drug candidates, other approaches that can penetrate bacterial cell membranes, improve inflow, block efflux, and target certain infections with therapeutic antibodies are appealing and encouraging. An effective arsenal to protect global health might be loaded into the antibiotic pipeline with the help of incentivised commercial models, government policies, and a defined regulatory framework.

Hussain, Naveed *et al*. (2024) [3]. The increasing prevalence of germs that are resistant to several drugs is a major concern for global public health since it reduces the effectiveness of traditional antibiotics and makes diseases more worse. The only way to overcome this problem is to create new and improved antimicrobials that can fight these resistant bacteria. The goal of this study is to determine whether or not new antimicrobial drugs have therapeutic potential by testing their effectiveness against MDR bacteria derived from clinical samples. Methods: The ethics board of Nishtar Medical University Multan approved this experimental investigation before it began. Multiple drug-resistant bacteria were found in bacterial strains that were extracted from different types of clinical samples. We used bactericidal assays and minimum inhibitory concentration (MIC) tests to measure the antimicrobial activity of three new medicines. These procedures measure the agents' capacity to suppress and eliminate bacterial growth. Findings: The new chemicals' effectiveness in killing the germs varied. With a bactericidal activity of 99.0%, Agent A significantly reduced Klebsiella *pneumoniae*. Agent B reduced Pseudomonas aeruginosa by 82.4%, while Agent C was particularly effective against Acinetobacter baumannii, with an MIC of 0.66 µg/ml. The medicines' strong antibacterial capabilities were shown by these findings across several MDR bacterial strains. In conclusion, this research shows that new antimicrobial medicines have great promise for treating illnesses caused by multidrug-resistant bacteria. These medicines may play a pivotal role in creating novel treatment approaches for resistant infections, according to the substantial bactericidal effects shown.

Qadri, Hafsa *et al*. (2020) [4]. Antimicrobial medications are the silver bullet that fight infectious illnesses, which disproportionately affect people in underdeveloped countries and put a huge strain on healthcare systems there. The problem of antimicrobial resistance (AMR), however, is a serious and pressing one on a worldwide scale. Emerging multidrug-resistant bacterial (e.g., tuberculosis, cholera) and fungal (e.g., candidiasis) illnesses have few antibiotic options, and their development has many root causes. Given the gravity of the problem, there is an immediate need to find, create, test, and advance new techniques and approaches that may be used to combat the rising tide of antimicrobial resistance. When it comes to fighting the problem of antimicrobial drug resistance, immunotherapy is a major weapon in the arsenal. Similarly, medication combination treatment is an additional viable option for extending the life of antimicrobials and slowing the spread of resistance. Another new treatment option for preventing the spread of MDR bacteria is bacteriophage therapy. In addition, CRISPR, a novel genome editing tool, has several potential uses in protecting host defences against various resistance threats. Along with our growing understanding of the many ways in which microbiological pathogens develop resistance to antimicrobial drugs, this article provides an overview of some of the more recent developments in the

fight against pathogenic microbes and microbial invasions, including combination therapy, immunotherapy, bacteriophage therapy, and CRISPR/Cas. Thus, in order to accomplish the aims of developing effective antimicrobial drugs and their targets, it is necessary to comprehend the various drug resistance mechanisms and new control plans/approaches. This knowledge will ultimately aid in reducing the problem of the growing threat of antimicrobial drug resistance in human pathogenic microbes.

O'Connell, Kieron *et al*. (2013) [12]. Around the middle of the twentieth century, efficient antibiotic treatments for infectious disorders were introduced, which greatly altered clinical procedures and paved the way for contemporary medicine. The number of people killed or crippled by bacterial infections dropped dramatically as many diseases that were formerly considered fatal were cured. It is hard to fathom a world without effective antibacterials, given their tremendous historical success; but, the unstoppable development of antibiotic resistance has brought this terrifying prospect to the forefront for some illnesses. As a result of both underinvestment in antibacterial research and the relentless selection for resistant bacteria, the effectiveness of current medicines has been steadily declining, and there is a dearth of new structural classes that may either replace or supplement them. Because of this, there is an immediate need to find new antibiotics and treatment methods; this will certainly be a major problem for medicinal chemists in the 21st century.

Materials and Methods

In vitro susceptibility testing in MDR bacterial species

Preparation of extracts: The cell-free supernatant from five-litre shake cultures of the most active isolates was split in three, with ethyl acetate and n-butanol each extracting one part. These were further dried down by evaporation in the rotary evaporator at lower pressure. The extracts were reconstituted in 100% DMSO at a concentration of 1 mg/mL and then passed through a 0.22 µm syringe filter. Antibiotics were purchased from pharmaceutical companies and diluted to a dosage of 1 mg/mL. Standard antibiotics or comparators used in these investigations were azithromycin, ampicillin, clindamycin, erythromycin, levofloxacin, linezolid, penicillin, and vancomycin.

Low-dose micro-dilution assays we tested the most effective extracts against a range of MDR bacteria and determined their minimum inhibitory concentrations (MICs). Using the micro-broth dilution technique in accordance with the recognised standard M7-A09 as suggested by the Clinical and Laboratory Standards Institute (2012), the following bacteria were tested: Streptococcus *pneumoniae*, Streptococcus *pyogenes*, *Enterococci* sp., methicillin resistant Staphylococcus aureus, and *Acinetobacter* sp. The two groups that acted as controls were sterile Mueller-Hinton broth that did not include any medication and sterile DMSO that was added to this broth.

Evaluation of antibacterial effectiveness *in vitro*: The effectiveness of a substance as an antibiotic and the pace at which it kills microorganisms may be determined by Time-Kill Kinetic investigations. Following the protocols laid forth by the CLSI, time-kill experiments were conducted using the macrobroth-dilution technique first described by Hoellman *et al*. (2003) [13]. Bacterial cell inoculum solutions containing around 105 CFU/mL of exponentially developing cells were used for this investigation. Inoculum suspensions with final concentrations of ½× MIC, MIC, 2× MIC, 4× MIC, and 8× MIC were mixed with 10 mL of the test drug. Every experiment had a growth control that consisted of the same bacterial strain but without the test chemical. The inoculum cultures were placed on an orbital shaker set at 200 rpm and incubated at 37 °C. Timed intervals of incubation (0, 1, 2, 4, 6, 8, 24, and 30 h) were used to extract aliquots from the inoculum culture, which were then diluted in saline serially as required. For time-kill experiments, the viable cell count (Log10 CFU/mL) was measured at 0, 1, 2, 4, 6, 8, 24, and 30 hours and compared to the 0 hour value. Placing 25 µL of each dilution on an MHA plate allowed the plate count method to determine the numbers of viable cells. We incubated all of the plates at 37 °C for 24 hours. The trials were carried out three times. By graphing the log10 colony forming unit per millilitre (CFU/mL) against time (hours), the data were analysed as killing curves, and the change in bacterial concentration was calculated. Bacteriostatic activity is defined as a decrease of less than or equal to 3 log10 CFU/mL relative to the initial inoculum, while bactericidal activity is defined as the lowest concentration that reduced the original inoculum by at least 3 log10 CFU/mL (99.9% killing) at each time point. A ciprofloxacin-resistant strain of S. *pneumoniae* (B-10350) was subjected to crude extracts (RK57EA and RK57nBE) at concentrations varying from 0.5 to 4 µg/mL (1×- 8×), while levofloxacin was used as a control at doses ranging from 8 to 32 µg/mL (1×-4×). The drug-sensitive strain of S. *pyogenes* 231 was subjected to doses ranging from 0.5 to 8 µg/mL (1×-16×) of the crude extracts (RK57EA and RK57nBE), with levofloxacin serving as a control. Gamma piraciformis ATCC 19434 At doses ranging from 1 to 16µg/mL (0.5×-8×), a vancomycin-susceptible strain was treated to the crude extracts (RK57EA and RK57nBE), with levofloxacin and vancomycin serving as the two controls. Levofloxacin and vancomycin concentrations varied between 4 and 8 µg/mL and 1 and 2 µg/mL, respectively, according to the 1×-2× scale.

Data Analysis

Microbiological drug resistance testing *in vitro*

Acinetobacter sp., methicillin-resistant Staphylococcus aureus (MRSA), Streptococcus *pneumoniae*, Enterococci sp., and Streptococcus *pyogenes* were tested for inhibitory activities in order to determine the susceptibility of bioactive crude extracts from rare actinomycetes. Their results were superior to those of the gold standard control medications in a number of tests.

Table 1: Antibacterial activities of the 13 promising isolates selected after secondary screening against Gram–positive bacterial pathogens.

Strain No.	Extracts	Test Organisms (inhibition zones measured as diameter in mm)							
		S. aureus 25923	*S. aureus* 29213	MRSA 562	MRSA MU50	*S. epidermidis* 12228	*E. faecalis* 29212	*C. difficile*	*S. pneumoniae*
RK1	EA	14	–	17	12	22	14	–	18
	ME	15	10	18	10	24	16	–	21
RK6	EA	20	19	15	23	27	21	–	13
	ME	18	21	–	27	30	24	–	15
RK43	EA	–	12	–	15	–	–	–	20
	ME	–	10	–	–	–	–	–	18
RK53	EA	15	17	20	28	32.5	19	18	18.5
	ME	20	20	17	21	32.5	18.5	24	22
RK54	EA	17	15	16	22	25	19	22	20
	ME	13	15	15	18	27	12	–	19
RK57	EA	14.5	15	13	14	27	14	15	14
	ME	17	15.5	12	15	23	15	20	–
RK59	EA	–	–	10	15	–	10	12	–
	ME	–	–	–	17	22	–	10	–
RK61	EA	17	15	20	12	30	17	16	12
	ME	22	13	17	10	27	12	19	10
RK2_63	EA	18	–	–	11	15	22	25	25
	ME	17	–	–	–	14	24	23	23
RK66	EA	30	14	22	24	23	12	13	15
	ME	28	11	20	23	21	13	–	17
RK71	EA	29	17	19	19	30	27	29	31
	ME	28	17	19	21	31	25	30	32
RK2_75	EA	18	17	20	24	30	15	14	17
	ME	15	18	19	21	30	13	10	21
RK79	EA	–	–	10	14	22	10	–	–
	ME	–	–	12	12	–	10	–	–

Table 2: Antibacterial activities of the 13 most potent isolates selected after secondary screening against Gram–negative bacterial pathogens.

Strain No.	Extracts	Test Organisms (inhibition zones measured as diameter in mm)				
		E. coli ACR	*E. coli* 25922	*H. influenzae* ACR	*Bacteroides fragilis*	*P. aeruginosa*
RK1	EA	14	–	16	27	–
	ME	11	–	20	31	–
RK6	EA	13	–	29	16	–
	ME	15	–	27	12	–
RK43	EA	–	–	21	–	–
	ME	–	–	18	–	–
RK53	EA	–	–	34	22	–
	ME	10	13	31	29	–
RK54	EA	16	18	31	32	–
	ME	13	19	32	36	–
RK57	EA	15	12	35	–	–
	ME	10	10	33	–	–
RK59	EA	–	–	17	13	17
	ME	–	10	–	10	19
RK61	EA	–	–	–	18	–
	ME	–	–	17	–	–
RK2_63	EA	19	–	30	–	–
	ME	16	–	27	–	–
RK66	EA	21	18	35	16	–
	ME	19	15	36	14	–
RK71	EA	22	16	37.5	25	–
	ME	24	15	35	27	–
RK2_75	EA	–	–	–	–	–
	ME	–	–	–	17	–
RK79	EA	10	–	29	–	–
	ME	–	–	30	10	–

Micro broth dilution assays for MIC

Anti-S. *pneumoniae* susceptibility testing and minimum inhibitory concentration (MIC) determination

The five strains' extracts, namely RK53, RK54, RK57, RK66, and RK71, demonstrated activity against multidrug-resistant S. *pneumoniae* strains with minimum inhibitory concentrations (MICs) ranging from 0.25 to more than 32 µg/mL. In fact, MICs for a few of them were as low as 0.125 µg/mL. According to Tables 3 and 6, the majority of these extracts showed moderate to excellent efficacy against both sensitive and multidrug-resistant S. *pneumoniae* strains. Both RK57EA and RK57nBE extracts had much

lower MICs, with MIC50 and MIC90 values of 0.5 µg/mL, respectively. Taken together, the RK53EA, RK57EA, and RK57nBE extracts showed strong action against several strains of S. *pneumoniae*, including those that are sensitive to penicillin, resistant to penicillin, erm (B), mef (E), and FQR. What's more, their minimum inhibitory concentrations (MICs) were much lower than those of the commonly used antibiotic levofloxacin. To far, there has been little investigation into the potential of actinomycete antimicrobial metabolites against drug-resistant S. *pneumoniae*. According to Ola *et al*. (2013) [14], cyclic depsipeptides enniatins B1 and A1, which were obtained by co-culturing the fungal endophyte Fusarium tricinctum with the bacterium Bacillus subtilis, exhibited activity against S. aureus, S. *pneumoniae*, and Enterococcus faecalis, with minimum inhibitory concentration (MIC) values ranging from 2 to 8 µg/mL. Plus, lateropyrone, which is derived from F. tricinctum, showed promising antibacterial effects against B. subtilis, S. aureus, S. *pneumoniae*, and E. faecalis, with MIC values ranging from 2-8 µg/mL. Out of all the purified natural products mentioned, the antibacterial activity of RK57EA and RK57nBE crude extracts (with MIC50 and MIC90 = 0.5 µg/mL) was determined to be the most powerful. When tested against penicillin-intermediate strains, these extracts showed no signs of diminished activity. Consistent with other studies (Mazzetti *et al*. 2012; Ola *et al*. 2013) [14, 15], we found that these bioactive compounds had a greater degree of antibacterial activity and were just as effective against multidrug-resistant clinical isolates of S. *pneumoniae*.

Table 3: MICs (µg/mL) for the microbial extracts and standard drugs against sensitive and penicillin–resistant S. *pneumoniae*.

S. no.	Organism	RK53 (EA)	RK53 (nBE)	RK54 (EA)	RK54 (nBE)	RK57 (EA)	RK57 (nBE)	RK66 (EA)	RK66 (nBE)	RK71 (EA)	RK71 (nBE)	Clindamycin	Erythromycin	Levofloxacin	Penicillin
1	155	1	8	>32	>32	0.25	0.25	>32	>32	32	8	0.015	0.03	0.5	0.03
2	B–1730	0.5	4	>32	>32	0.25	0.25	>32	>32	32	8	0.03	0.03	2	0.5
3	ATCC P–IR 49619	0.5	8	>32	>32	0.5	0.5	>32	>32	>32	>32	0.06	0.03	1	0.5
4	PR AB17	0.5	8	>32	>32	0.5	0.5	>32	>32	32	>32	0.03	0.015	0.5	>4
5	PR AB 16	0.25	8	>32	>32	0.25	0.25	>32	>32	32	>32	0.06	0.03	1	>4
6	PR AB 21	0.25	4	>32	>32	0.5	0.25	>32	>32	32	>32	0.03	0.03	2	2
7	P–IR AB11	0.5	4	>32	>32	0.25	0.25	>32	>32	>32	4	0.03	0.015	0.5	1

P–IR = Penicillin intermediate resistant; PR = Penicillin resistant.

Table 4: MICs (µg/mL) for the microbial extracts and standard drugs against erm (B) S. *pneumoniae*

S. no.	Organism	RK53 (EA)	RK53 (nBE)	RK54 (EA)	RK54 (nBE)	RK57 (EA)	RK57 (nBE)	RK66 (EA)	RK66 (nBE)	RK71 (EA)	RK71 (nBE)	Clindamycin	Erythromycin	Levofloxacin	Penicillin
1	PR 3390	1	8	>32	>32	1	0.5	>32	>32	32	>32	>4	>4	0.5	2
2	P-IR MR757	0.5	4	>32	>32	0.13	0.25	>32	>32	32	8	>4	>4	1	1
3	P-IR MA80	0.5	4	>32	>32	0.25	0.25	>32	>32	>32	32	>4	>4	1	1
4	1188	1	8	>32	>32	0.5	0.5	>32	>32	32	32	>4	>4	0.5	0.25
5	3968	0.5	4	>32	>32	0.25	0.25	>32	>32	32	4	2	>4	0.5	0.25
6	1275	0.5	8	>32	>32	0.5	0.5	>32	>32	32	>32	>4	>4	1	0.03
7	1256	0.25	8	>32	>32	0.25	0.5	>32	>32	32	>32	>4	>4	0.5	4
8	3565	0.5	8	>32	>32	0.5	0.5	>32	>32	32	>32	>4	>4	0.5	0.03
9	3579	0.5	8	>32	>32	0.5	0.5	>32	>32	32	>32	>4	>4	0.5	0.125
10	2840	0.25	4	>32	>32	0.25	0.25	>32	>32	32	32	4	4	0.5	0.125
11	217	0.5	8	>32	>32	0.5	0.5	>32	>32	>32	>32	>4	>4	0.5	0.03
12	3271	0.5	4	>32	>32	0.25	0.25	>32	>32	32	32	>4	>4	1	1

P–IR = Penicillin intermediate resistant; PR = Penicillin resistant.

Table 5: MICs (µg/mL) for the microbial extracts and standard drugs against mef (E) S. *pneumoniae.*

S. no.	Organism	RK53 (EA)	RK53 (nBE)	RK54 (EA)	RK54 (nBE)	RK57 (EA)	RK57 (nBE)	RK66 (EA)	RK66 (nBE)	RK71 (EA)	RK71 (nBE)	Clindamycin	Erythromycin	Levofloxacin	Penicillin
1	Peni-IR 3583	0.5	8	>32	>32	0.5	0.5	>32	>32	32	>32	0.03	4	0.5	2
2	PR 3404	0.5	8	>32	>32	0.5	0.5	>32	>32	>32	>32	0.06	>4	1	>4
3	PR 994	1	8	>32	>32	0.5	0.5	>32	>32	32	8	0.03	2	0.5	4
4	PR AB 34	0.5	8	>32	>32	0.5	0.5	>32	>32	>32	>32	0.06	>4	2	>4
5	PR 4748	2	16	>32	>32	2	2	>32	>32	>32	8	0.125	>4	2	4
6	5055	0.25	4	>32	>32	0.25	0.25	>32	>32	32	>32	0.03	4	0.5	0.03
7	5089	0.5	8	>32	>32	0.5	0.5	>32	>32	32	>32	0.03	4	1	4.75
8	1251	0.5	8	>32	>32	0.5	0.5	>32	>32	>32	>32	0.03	4	0.5	0.5
9	1294	0.5	8	>32	>32	0.25	0.25	>32	>32	32	>32	<0.008	>4	0.5	0.03
10	3264	1	4	>32	>32	0.13	0.125	>32	>32	32	16	0.5	>4	0.5	0.25
11	B739	0.25	4	>32	>32	0.25	0.25	>32	>32	32	16	0.03	0.03	2	0.5

P–IR = Penicillin intermediate resistant; PR = Penicillin resistant.

Table 6: MICs (µg/mL) for the microbial extracts and standard drugs against FQR S. *pneumoniae*

S. no.	Organism	RK53 (EA)	RK53 (nBE)	RK54 (EA)	RK54 (nBE)	RK57 (EA)	RK57 (nBE)	RK66 (EA)	RK66 (nBE)	RK71 (EA)	RK71 (nBE)	Clindamycin	Erythromycin	Levofloxacin	Penicillin
1	622286	1	8	>32	>32	0.5	0.5	>32	>32	32	16	>4	>4	8	0.03
2	TPS 3	0.5	4	>32	>32	0.25	0.25	>32	>32	32	2	0.03	4	8	4
3	2304	1	8	>32	>32	0.5	0.5	>32	>32	32	4	0.03	0.015	>8	0.03
4	1629 2nd	0.5	4	>32	>32	0.25	0.25	>32	>32	32	8	0.06	0.03	8	0.03
5	214152	1	8	>32	>32	0.25	0.25	>32	>32	32	4	0.03	0.03	>16	0.03
6	402123	1	8	>32	>32	0.25	0.25	>32	>32	>32	8	0.03	0.03	16	0.03
7	502226	0.5	4	>32	>32	0.5	0.5	>32	>32	32	8	0.015	0.015	16	0.03
8	723084	0.5	4	>32	>32	0.5	0.5	>32	>32	>32	4	0.03	0.03	16	0.03
9	B-10350	1	8	>32	>32	0.5	0.5	>32	>32	32	4	0.03	0.03	8	0.03

Finding the minimum inhibitory concentrations and patterns of sensitivity to S. *pyogenes*

Tables 7 and 8 show that against both sensitive and multidrug-resistant S. *pyogenes* strains, the majority of the extracts showed moderate to excellent efficacy. In terms of killing multidrug-resistant S. *pyogenes* strains, these extracts exhibited MICs that varied between 1 and more than 32 µg/mL. Even as low as 0.5 µg/mL, several of them exhibited MICs. When tested against isolates of Staphylococcus *pyogenes* that were susceptible to or resistant to erythromycin (because of the erm (A), erm (B), or mef (E) genes), RK57EA and RK57nBE had much lower MICs. Both of these extracts have MIC50 and MIC90 values of 1-2 µg/mL, respectively. The antibacterial activity of RK53EA, RK57EA, RK57nBE, and RK71nBE extracts was strong against MDR S. *pyogenes* strains, and their minimum inhibitory concentrations (MICs) were comparable to levofloxacin's. Research on the antibacterial properties of natural compounds against Staphylococcus *pyogenes* is still in its early stages. As per Seanego and Ndip (2012) [16], the minimum inhibitory concentration (MIC) for S. *pyogenes* against methanol extracts of Garcinia kola seeds was 40 µg/mL. A novel antimicrobial natural compound (1) was shown to have action against S. *pyogenes* with a minimum inhibitory concentration (MIC) of 4 µg/mL after being extracted and studied from the Hypericum olympicum L. cf. uniflorum plant (Shiu *et al*. 2013) [17]. Out of all the pure natural products tested, the antibacterial activity of RK57EA and RK57nBE extracts (MIC50 and MIC90 = 1-2 µg/mL, respectively) was determined to be the most powerful. Confirming a greater degree of antimicrobial susceptibility, these results are similar with ours as well as those previously published (Seanego and Ndip 2012; Shiu *et al*. 2013) [16, 17]. Furthermore, the data show that these extracts had no influence on the macrolide resistance patterns of any of the S. *pyogenes* strains tested.

Table 7: MICs (µg/mL) for the microbial extracts and comparators against sensitive S. *pyogenes*

S. no.	Organism	RK53 (EA)	RK53 (nBE)	RK54 (EA)	RK54 (nBE)	RK57 (EA)	RK57 (nBE)	RK66 (EA)	RK66 (nBE)	RK71 (EA)	RK71 (nBE)	Clindamycin	Erythromycin	Levofloxacin
1	228	2	16	>32	>32	1	1	>32	>32	32	4	0.03	0.03	0.5
2	229	1	16	>32	>32	1	1	>32	>32	32	4	0.03	0.03	0.5
3	231	2	16	>32	>32	0.5	0.5	>32	>32	>32	4	0.03	0.03	1
4	M1633B	2	16	>32	>32	0.5	0.5	>32	>32	32	4	1	0.03	1
5	SS19B	2	16	>32	>32	0.5	0.5	>32	>32	32	4	0.03	0.03	1
6	ATCC 12344	1	16	>32	>32	1	1	>32	>32	32	4	>16	>16	0.5
7	ATCC 19615	1	8	>32	>32	0.5	0.5	>32	>32	32	4	2	0.125	0.5
8	ATCC25147	2	8	>32	>32	0.5	0.5	>32	>32	>32	8	1	<0.015	0.5
9	234	2	16	>32	>32	0.5	0.5	>32	>32	32	4	2	0.03	1
10	20361	1	8	>32	>32	0.5	0.5	>32	>32	32	8	4	0.06	1
11	203C	1	8	>32	>32	1	1	>32	>32	>32	4	2	1	0.5
12	19784	2	8	>32	>32	1	1	>32	>32	>32	4	4	0.06	1
13	217	1	16	>32	>32	1	1	>32	>32	32	8	2	0.03	1
14	223	2	8	>32	>32	1	1	>32	>32	32	8	4	0.03	1
15	226	1	16	>32	>32	1	1	>32	>32	32	4	2	0.03	1
16	232	2	8	>32	>32	1	1	>32	>32	>32	4	4	0.06	0.5
17	238	2	8	>32	>32	1	1	>32	>32	>32	4	4	0.06	1
18	239	1	16	>32	>32	0.5	0.5	>32	>32	32	8	2	0.03	1
19	263	2	16	>32	>32	1	1	>32	>32	>32	4	4	0.03	1
20	267	2	16	>32	>32	1	1	>32	>32	>32	4	2	0.03	1

Table 8: MICs (µg/mL) for the microbial extracts and comparators against sensitive S. *pyogenes*

S. no.	Organism	RK53 (EA)	RK53 (nBE)	RK54 (EA)	RK54 (nBE)	RK57 (EA)	RK57 (nBE)	RK66 (EA)	RK66 (nBE)	RK71 (EA)	RK71 (nBE)	Clindamycin	Erythromycin	Levofloxacin
21	276	1	8	>32	>32	1	1	>32	>32	32	4	2	0.03	0.5
22	293	1	16	>32	>32	1	1	>32	>32	32	4	2	0.06	1
23	294	2	8	>32	>32	1	1	>32	>32	32	8	2	0.03	1
24	298	2	8	>32	>32	0.5	0.5	>32	>32	32	4	2	0.06	1
25	288	2	8	>32	>32	0.5	0.5	>32	>32	32	4	4	0.06	1
26	269	2	8	>32	>32	1	1	>32	>32	>32	8	4	1	1
27	254	1	8	>32	>32	1	1	>32	>32	32	4	4	0.125	0.5
28	297	1	8	>32	>32	1	1	>32	>32	>32	8	2	0.125	1

In addition to these naturally occurring substances, a new class of acylides was developed to counteract the effects of macrolides. These acylides showed remarkable efficacy against strains of S. *pyogenes* that had developed resistance to macrolides, with minimum inhibitory concentrations (MICs) ranging from 0.125 to 1 µg/mL (Pandya *et al*. 2010) [18]. The findings of our study are consistent with prior investigations as the MIC50 and MIC90 values for RK57EA and RK57nBE were 1 and 2 µg/mL, respectively. These findings demonstrate that the extracts have potent antibacterial properties, on par with those of previously isolated and manufactured antimicrobial substances.

Conclusions

Further investigation into the mechanisms of action included determining the *in vitro* antimicrobial potencies of the most promising extracts using *in vitro* cell-free transcription/translation of the luciferase gene utilising S30 bacterial extract and TNT human ribosome. Much lower quantities of RK57EA and RK57nBE were needed to block bacterial ribosomes compared to mammalian ribosomes, according to the cell free transcription and translation experiment. There was no evidence of membrane disruption activity in the BacLight bacterial viability kit bacterial viability assay when both of these extracts were tested. Rare actinomycetite metabolites have a novel method of action: they block protein synthesis, and this is the first description of their bactericidal ability against multidrug-resistant bacteria. On the other hand, RK53 extracts showed a "less crowded" compound pattern, making it the second most promising isolation after RK57. Deferoxamine, ferrioxamine, and prodigiosin C25 are among the chemicals it was discovered to make. Curiously, most of the metabolites from both of these isolates did not provide any hits from the Chapman & Hall database when we ran a straight mass-based search, which might indicate that these are some new chemicals. Ongoing research aims to characterise the active principle chemicals derived from their extracts that exhibit good to exceptional bactericidal action. Purified versions of these compounds have the potential to combat multidrug-resistant bacteria due to their high *in vitro* effectiveness and potency. The two most promising extracts, RK57EA and RK57nBE, include bioactive chemicals that might be useful in pharmaceutical formulations, although more research is required to identify and isolate these molecules.

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