



Antimicrobial Susceptibility Profile of Market-Sold Synthetic Antibiotic Discs Against *Bacillus subtilis* and *Klebsiella pneumoniae*

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Abstract

In the 21st century, one of the major global health challenges is the increasing threat of antimicrobial resistance, largely resulting from the overuse and misuse of antibiotics in both clinical and community settings. Seven (7) synthetic antibiotic drugs—namely Augmentin, amoxicillin, ciprofloxacin, cefotaxime, erythromycin, gentamicin, and levofloxacin—were obtained from a laboratory store, Ado Jone Limited, Kano. Two clinical isolates, *Bacillus subtilis* and *Klebsiella pneumoniae*, were obtained from Manzanita Research Laboratory, located behind Opeyemi Filling Station, Kwara State. Mueller–Hinton agar was prepared and poured into forty-two (42) sterile Petri dishes. A saline solution was prepared to dilute the bacterial isolates, and the turbidity was adjusted to match the 0.5 McFarland standard. A sterile cotton swab was used to streak the diluted organisms evenly on the surface of the agar plates. Antibiotic discs were then placed on the inoculated plates alongside a control disc consisting of sterile filter paper soaked in distilled water. All inoculated plates were incubated at room temperature, and after 24 hours, the diameters of the zones of inhibition were measured using a vernier caliper. All tests were carried out in triplicate. The highest antibacterial activity against *Bacillus subtilis*, with a mean inhibition zone of 27.17 mm, was demonstrated by erythromycin, followed by levofloxacin (21.07 mm). Gentamicin (18.63 mm), cefotaxime (17.67 mm), and ciprofloxacin (17.63 mm) showed lower levels of activity. Augmentin produced no inhibition (0.00 mm), indicating resistance of *B. subtilis* to the antibiotic. For *Klebsiella pneumoniae*, levofloxacin recorded the highest activity with a mean inhibition zone of 45.87 mm, followed by gentamicin (33.10 mm) and amoxicillin (29.10 mm). Augmentin showed moderate inhibition (23.10 mm), while ciprofloxacin (19.40 mm) and cefotaxime (18.10 mm) exhibited the lowest antibacterial effects

Keywords: *Bacillus Subtilis*, *Klebsiella Pneumonia*, Antibiotic, Inhibition Zone, Levofloxacin

Introduction

In the 21st century, one of the top global health issues is the increasing threat of antimicrobial resistance, primarily due to the overuse and misuse of antibiotics in both clinical and community settings. (Mączyńska et al., 2023). Antimicrobials are substances that inhibit the growth or kill microorganisms like bacteria, fungi, and viruses (Martinez et al., 2019) mostly by interfering with outer membrane synthesis of the bacteria this is because LPC-233 is a chemical inhibitor of the LpxC enzyme necessary for biosynthesis of the lipid A component of Gram-negative bacteria. This inhibition destabilizes the bacterial outer membrane, and death ensues rapidly. LPC-233 is effective against 285 resistant strains and is stable in vivo, indicating it may be an excellent treatment for urinary tract infections. (Duke University, 2023), secondly inhibition of protein synthesis by ribosomal binding, Scientists at Harvard University identified cresomycin as one of the most active ribosome-binding agents against bacteria; it also binds to resistant organisms such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Generally, Binding has an enormous inhibitory effect on protein synthesis, with increased activity over established antibiotics. (Harvard University, 2024), another mechanism is targeting bacterial virulence factor as Current research has focused on synthetic virulence characteristics inhibitors rather than inhibiting bacterial growth directly. By targeting processes such as toxin production and biofilm formation, these inhibitors reduce pathogenicity and the possibility of resistance development (Harvard University, 2024), another way in which this antibiotic inhibit the growth of the microorganism is membrane disruption mimicking host defense peptide, Brilacidin is a synthetically prepared arylamide foldamer that

replicates the amphiphilic character of antimicrobial peptides. It kills the bacterial membrane resulting in cell death and possesses minimal cytotoxicity against mammalian cells. Its mechanism reduces resistance development potential. (Wikipedia contributors,2024), and lastly development of Teixobactin, teixobactin analogs with non-proteogenic amino acids have also been synthesized by scientists, which are more effective against drug-resistant biofilms and bacteria. The analogs have potential for the therapy of chronic infections, including surgical site infections and cystic fibrosis. (University of Liverpool,2024).

According to the World Health Organization (WHO, 2021), antimicrobial resistance has the potential to render existing treatments obsolete. making it an emerging problem that results in prolonged infections, increased morbidity, and higher mortality (Chaisaeng et al., 2024). The cornerstone of antimicrobial therapy, which includes synthetic drugs such as β -lactams, fluoroquinolones, aminoglycosides, and macrolides, is at the verge of losing there efficacy due to a rise in resistant pathogenic bacteria (Zhang et al., 2021).Two unique species for the purpose of antimicrobial susceptibility testing are *Bacillus subtilis* and *Klebsiella pneumoniae*. *B. subtilis*, as a ubiquitous non-pathogenic probiotic bacterium and soil organism, is widely used as an antimicrobial model system because of its highly characterized genetic system as well as its reproducible susceptibility to the majority of antibiotics (Daneshazari et al., 2023). On the other hand, *K. pneumoniae* is a significant Gram-negative bacterium implicated in nosocomial infections, including pneumonia, bacteremia, and urinary tract infections. Its β -lactam, fluoroquinolone, and aminoglycoside resistance, particularly by extended-spectrum β -lactamases (ESBLs) and carbapenemases, has been one of the driving factors for worldwide AMR problems (Cireșă et al., 2024; Worku et al., 2024). *K. pneumoniae* can readily carry multiple resistance genes and render most of the drugs being used ineffective. Surveillance studies that have been carried out in the recent past have found elevated levels of resistance against β -lactam antibiotics and aminoglycosides (Worku et al., 2024). For instance, Okwuonu & Chukwura (2022) reported that *K. pneumoniae* isolates were sensitive to amoxicillin/clavulanate with inhibition zones ≥ 18 mm, but resistance was observed at ≤ 13 mm. Similarly, the same study indicated a cefotaxime sensitivity rate of ≥ 26 mm and resistance at ≤ 22 mm, demonstrating a moderate susceptibility among tested isolates.

Along with these results, ciprofloxacin and gentamicin susceptibility to *K. pneumoniae* was also shown in another Peshawar study with clear disk diffusion breakpoints: ≥ 26 mm for strains sensitive to ciprofloxacin and ≥ 15 mm for gentamicin-sensitive isolates (Khan et al., 2025). Worku et al. (2024) reported cefotaxime susceptibility with inhibition zones of 18 to 22 mm, while Khan et al. (2025) reported ciprofloxacin susceptibility of 20 to 23 mm, although strains resistant to it with a zone of merely 12 mm have also been reported. Levofloxacin and erythromycin were found to be intermediate in activity with inhibition zones of 16 to 21 mm and 14 to 18 mm, respectively (Zhang et al., 2021; Akhtar et al., 2023). These findings are not only the result of variable efficacy of antibiotic classes but also the pressing matter of limiting therapeutic options against *K. pneumoniae*. Despite such inhibitions, trends towards middle and resistant types illustrate an emerging issue in the therapeutic management of *Klebsiella* infections.

Conversely, *Bacillus subtilis*, a Gram-positive bacterium with endospore production, is quite renowned for both its industrial application and environmental sustainability (Parulekar et al., 2019). While generally non-pathogenic, it is also exploited as a model organism for screening against antimicrobials owing to its clearly defined sensitivity towards a broad range of antibiotics (Urme et al., 2023). Conscientiabeam Archive (2023) research reported that gentamicin and erythromycin inhibited the growth of *B. subtilis* with inhibition zones of approximately 32 to 35 mm and 34 to 36 mm, respectively. Amoxicillin (30 μ g) was moderately inhibitory, with inhibition zones of 22 ± 0.6 mm. For instance, gentamicin exhibited a mean zone of inhibition of 21 to 25 mm against *B. subtilis* (Daneshazari et al., 2023), while ciprofloxacin exhibited 25 to 27 mm inhibition zones against the same bacterium (Akhtar et al., 2023). All these results indicate *B. subtilis* as a good bioindicator organism for the assessment of antibiotic potency.

Despite the growing number of publications on AMR, little direct comparison of the antimicrobial susceptibility patterns of commercial synthetic antibiotic discs offered for sale in local markets, particularly in resource-poor settings, has been reported. Comparative testing of antibiotics such as amoxicillin, augmentin, cefotaxime, ciprofloxacin, gentamicin, levofloxacin, and erythromycin against *B. subtilis* and *K. pneumoniae* is informative in terms of both model organisms and clinically relevant pathogens. The aim of this study, therefore, is to determine the antimicrobial susceptibility pattern of specific synthetic antibiotic discs against *Bacillus subtilis* and *Klebsiella pneumoniae* with a focus on the zones of inhibition formed under standard conditions.

Materials and Methods

Collection of samples

Selection of Antimicrobial Agents

Synthetic Antimicrobial Agents, which are Standard rings of antibiotic discs containing Amoxicillin, Ciprofloxacin, Levofloxacin, Erythromycin, Gentamicin, Augmentin, and Cefotaxim, were purchased from the laboratory equipment store (Ado Jones Limited) located at 13 Ibrahim Taiwo Road, Kano State. The discs were obtained from and used according to Clinical Laboratory Standard Institute guidelines (CLSI, 2021).

Collection of bacterial strains

Gram-positive *Bacillus subtilis* and gram-negative *Klebsiella pneumoniae* bacteria were obtained from a standard certified microbiological laboratory, the Manzanita Research and Diagnostic Laboratory, located behind Opeyemi filling station, Gano, Kwara state. on 18th April 2025 and the isolates were confirmed by coagulase, catalase, and indole tests, accordingly. The isolates were further subcultured in peptone water and stored until analysis.

Media Preparation

500 mL of Mueller-Hinton Agar (MHA) was prepared by dissolving 19 grams in 500 mL of distilled water. The medium was sterilized by autoclaving at 121°C for 15 minutes and poured into 42 sterile Petri dishes under aseptic conditions. Mueller-Hinton Agar (MHA) is recommended for antimicrobial susceptibility testing due to its reproducibility and lack of antagonistic substances (CLSI, 2021).

Preparation of McFarlan standard turbidity:

To ensure a standardized microbial load for inoculation, a 0.5 McFarland turbidity standard was prepared. 1% solution of anhydrous barium chloride (BaCl₂) was prepared by dissolving 1 gram of BaCl₂ in 10 mL of distilled water, and 1% solution of sulfuric acid (H₂SO₄) was prepared by mixing 9 mL of distilled water with 1 mL of concentrated H₂SO₄. Approximately 9.95 mL of 1% sulfuric acid was measured into a test tube. Using a micro pipette, 0.05 mL of 1% barium chloride solution was added to the test tube containing 9.95 mL of 1% sulfuric acid. The mixture was combined and completely mixed (i.e, the barium chloride and sulfuric acid solutions) to form a turbid suspension. (Aryal, 2021), The tube was wrapped in aluminum foil to protect it from light and stored at room temperature for future use. (Headley *et al.*, 2020).

Antimicrobial Susceptibility Testing

Preparation of saline water for bacterial suspension

Saline water was prepared by dissolving 0.425 grams of sodium chloride, which was measured with an analytical balance, in 50 mL of distilled water, and pouring 10 mL each into four culture tubes. The two (2) organisms that were grown on petri dishes were swabbed and diluted in each of the culture tubes that is one organism in each tube, and were labelled. (Headley *et al.*, 2020).

Disc Diffusion Method

Mueller-Hinton agar plates were inoculated with standardized bacterial suspensions using sterile cotton swabs. The rings of Antibiotic discs for both gram-positive and gram-negative bacteria containing Amoxicillin, Ciprofloxacin, Levofloxacin, Erythromycin, Gentamicin, Augmentin, and Cefotaxime were placed on the surface of the agar using sterile forceps. The plates were incubated at 37°C for 24 hours. Zone diameters were measured and interpreted according to CLSI breakpoints (CLSI, 2021).

For the antibiotic disc, sterile distilled water was used as the control by soaking a sterile filter paper disc in the water before placing it on an inoculated plate to confirm that the zones of inhibition are a result of the antibiotic and not due to the moisture on the disc

Determination of Antimicrobial Activity

Post-incubation, zones of inhibition were measured in millimeters using a vernier caliper. The measurements were taken from the edge disc to the edge of the clear zone. Each test was performed in three biological replicates, in which each replicate had the average for each treatment (Wu *et al.*, 2024).

Statistical Analysis

The values obtained from the antimicrobial activity test were recorded as the mean zone of inhibition (mm) + standard deviation for every treatment. inhibition (mm) for every treatment. Calculation of the mean values was performed on Microsoft Excel 2019

Results

Table I shows the antimicrobial effects of selected synthetic antibiotics, namely Augmentin, Ciprofloxacin, Cefotaxime, Erythromycin, Gentamycin, and Levofloxacin, against *Bacillus subtilis*. The highest activity, with a mean inhibition zone of 27.17mm, was demonstrated by erythromycin. then amoxicillin 22.43 mm followed by Levofloxacin with an inhibition zone of 21.07mm, while gentamycin 18.63mm, cefotaxime 17.67mm, and ciprofloxacin 17.63mm demonstrated lower levels of activity. Augmentin produced no inhibition 0.00mm, indicating resistance of *B. subtilis* to it. These results suggest that erythromycin was the most effective synthetic antibiotic against *B. subtilis* in this study.

Table 1: Antimicrobial effects of Augmentin, ciprofloxacin, cefotaxime, erythromycin, gentamycin, and levofloxacin against a Gram-positive clinical isolate (*Bacillus subtilis*) indicated by zones of inhibition

Synthetic antimicrobials tested on a clinical isolate	Zones of inhibition in triplicate			Mean \pm SD (mm)
	Plate 1	Plate 2	Plate 3	
Augmentin	0.00	0.00	0.00	0.00 \pm 0.00
Ciprofloxacin	17.70mm	17.70mm	17.50mm	17.63 \pm 0.12
Cefotaxime	17.70mm	17.70mm	17.60mm	17.67 \pm 0.06
Erythromycin	27.20mm	27.20mm	27.10mm	27.17 \pm 0.06
Gentamycin	18.70mm	18.70mm	18.50mm	18.63 \pm 0.12
levofloxacin	21.10mm	21.10mm	21.00mm	21.07 \pm 0.
Amoxicillin	22.50mm	22.30mm	22.50mm	22.43 \pm 0.12
Control	0.00	0.00	0.00	0.00 \pm 0.00

Table 2 presents the antimicrobial effects of synthetic antibiotics against *Klebsiella pneumoniae*. Levofloxacin recorded the highest activity with a mean inhibition zone of 45.87 mm, followed by gentamycin (33.10 mm) and amoxicillin (29.10 mm). Augmentin showed moderate inhibition (23.10 mm), while ciprofloxacin (19.40 mm), erythromycin 18.97 mm and cefotaxime (18.10 mm) displayed the lowest effects. These findings indicate that *K. pneumoniae* was most susceptible to levofloxacin and gentamycin, while exhibiting reduced sensitivity to ciprofloxacin, erythromycin and cefotaxime.

Table 2: Antimicrobial effects of Augmentin, ciprofloxacin, cefotaxime, amoxicillin, gentamycin, and levofloxacin against Gram-negative clinical isolate (*Klebsiella pneumoniae*) indicated by zones of inhibition

Synthetic antimicrobials tested on a clinical isolate	Zones of inhibition in triplicate			Mean \pm SD (mm)
	Plate 1	Plate 2	Plate 3	
Augmentin	23.10mm	23.1mm	23.10mm	23.10 \pm 0.00
Ciprofloxacin	19.40mm	19.40mm	19.40mm	19.40 \pm 0.00
Cefotaxime	18.10mm	18.10mm	18.10mm	18.10 \pm 0.00
amoxicillin	29.10mm	29.10mm	29.10mm	29.10 \pm 0.00
Gentamycin	33.10mm	33.10mm	33.10mm	33.10 \pm 0.00
levofloxacin	46.20mm	45.70mm	45.70mm	45.87 \pm 0.29
Erythromycin	18.90mm	19.00mm	19.00mm	18.97 \pm 0.06
Control	0.00	0.00	0.00	0.00 \pm 0.00

Table 3 presents the average zones of inhibition (in millimeters) for seven synthetic antimicrobials against two bacterial isolates, *Bacillus subtilis* and *Klebsiella pneumoniae*. Larger zones indicate stronger antibacterial effects, while 0.00 mm indicates no inhibition. Levofloxacin was the most effective drug, producing the largest inhibition zones for *K. pneumoniae* (45.87 mm). *B. subtilis* was highly inhibited by Erythromycin (27.17 mm) and Levofloxacin (21.07 mm). Augmentin showed no activity (0.00 mm). *K. pneumoniae* was highly sensitive to Levofloxacin (45.87 mm) and Gentamycin (33.10 mm). Moderate sensitivity to Amoxicillin (29.10 mm) and Augmentin (23.10 mm). Augmentin was *B. subtilis*

Table 3: The average zones of inhibition as a result of antimicrobial effects of synthetic antimicrobials against *Bacillus subtilis* and *Klebsiella pneumoniae*

Synthetic antimicrobials tested on a clinical isolate	Average zones of inhibition for clinical isolate	
	<i>B. subtilis</i>	<i>K. pneumonia</i>
Augmentin	0.00 ± 0.00	23.10 ± 0.00
Ciprofloxacin	17.63 ± 0.12	19.40 ± 0.00
Cefotaxime	17.67 ± 0.06	18.10 ± 0.00
Erythromycin	27.17 ± 0.06	18.97 ± 0.06
Gentamycin	18.63 ± 0.12	33.10 ± 0.00
levofloxacin	26.97 ± 0.06	40.37 ± 0.58
Amoxicillin	22.43 ± 0.12	18.13 ± 0.46

Discussion

The present study assessed the antimicrobial activities of seven synthetic drugs namely; amoxicillin, augmentin, cefotaxime, ciprofloxacin, erythromycin, gentamicin, and levofloxacin against two model bacterial strains, *Bacillus subtilis* (Gram-positive) and *Klebsiella pneumoniae* (Gram-negative), using the disc diffusion method. The results revealed remarkable differences in susceptibility between the two organisms, as expected from differences in their cell wall structures and resistance patterns. Amongst the antibiotics screened, *Bacillus subtilis* was found to be most sensitive towards erythromycin with a mean inhibition diameter of 27.17 ± 0.06 mm, then amoxicillin 22.43 ± 0.12 followed by levofloxacin 21.07 ± 0.06 mm. Gentamicin, cefotaxime, and ciprofloxacin were found to have moderate inhibition diameters of 18.63 ± 0.12 mm, 17.67 ± 0.06 mm, and 17.63 ± 0.12 mm, respectively, while augmentin was not inhibitory, 0.00 ± 0.00 mm even the control disc showed no inhibition zone. These findings are consistent with previous reports of the strong inhibitory action of macrolides and fluoroquinolones against *B. subtilis*. Conscientiabeam Archive (2023) and Daneshazari et al. (2023) had previously reported 32 to 36 mm and 21 to 25 mm inhibition zones for erythromycin and gentamicin, respectively, consistent with the strong erythromycin activity in this study. Akhtar et al. (2023) had also recorded ciprofloxacin inhibition zones of 25 to 27 mm against *B. subtilis*, to which the present observation is nearest (17.63 mm), considering strain variation and test conditions.

The high sensitivity of *B. subtilis* to erythromycin may be due to good binding to the 50S ribosomal subunit, inhibiting the synthesis of proteins. Inhibition insensitivity by augmentin may be a sign of intrinsic resistance, possibly due to the organism being β -lactamase-independent, resistant to β -lactams, or due to low permeability of the cell wall to clavulanate combinations. This also agrees with the earlier findings that *Bacillus* species usually have variable susceptibility to β -lactams (Urme et al., 2023).

Klebsiella pneumoniae possessed a peculiar pattern of susceptibility as compared to *B. subtilis*. Levofloxacin produced the highest zone of inhibition, 45.87 ± 0.29 mm, followed by gentamicin, 33.10 ± 0.00 mm, and amoxicillin, 29.10 ± 0.00 mm. Augmentin produced moderate inhibition, 23.10 ± 0.00 mm, while ciprofloxacin, 19.40 ± 0.00 mm, erythromycin, 18.97 ± 0.06 mm, and cefotaxime, 18.10 ± 0.00 mm, produced the lowest activity.

These results are partially in agreement with Okwuonu & Chukwura (2022), who reported *K. pneumoniae* sensitivity to amoxicillin-clavulanate with inhibition zones of ≥ 18 mm and sensitivity to cefotaxime of ≥ 26 mm. The reduced activity of cefotaxime in this work, 18.10 mm, may reflect partial resistance or the emergence of extended-spectrum β -lactamases (ESBLs) that are capable of hydrolyzing third-generation cephalosporins. Similarly, Khan et al. (2025) have documented ciprofloxacin inhibition zones of 20 to 23 mm in sensitive strains, very much akin to the present observation of 19.40 mm.

The greater potency of levofloxacin 45.87 mm agrees with Zhang et al. (2021) and Akhtar et al. (2023), who attributed superior fluoroquinolone activity against Gram-negative isolates due to enhanced penetration and strong binding ability against DNA gyrase and topoisomerase IV. The strong activity of gentamicin 33.10 mm

also agrees with previous work of *Worku et al.* (2024), which reported 30 to 34 mm aminoglycoside inhibition zones against non-ESBL-producing *Klebsiella* isolates.

The relatively small inhibition zones of cefotaxime and ciprofloxacin suggest resistance development, possibly due to chromosomal mutation in *gyrA* and *parC* genes or plasmid-mediated β -lactamase production. The moderately high activity of amoxicillin (29.10 mm) can result from differences in the expression level of β -lactamases of the strains tested.

The Gram-negative *K. pneumoniae* was more sensitive to gentamicin and levofloxacin, but the Gram-positive *B. subtilis* was more sensitive to erythromycin. This is structural: Gram-negative bacteria possess an outer membrane that prevents some antibiotics from entering, but Gram-positive organisms lack an outer membrane, so they are more susceptible to drugs that interfere with peptidoglycan synthesis and protein translation (Zhang et al., 2021; *Worku et al.*, 2024). The lack of activity of augmentin against *B. subtilis* but moderate effectiveness against *K. pneumoniae* indicates that the β -lactamase inhibition of clavulanic acid is of more practical importance in Gram-negative situations where hydrolysis mediated by β -lactamases is an important resistance mechanism.

Conclusion

Current studies demonstrate vulnerability to be antibiotic-class and not broadly organism-dependent. *Bacillus* isolates would likely have large inhibition zones to macrolides and aminoglycosides (erythromycin, gentamicin), while many clinical *Klebsiella pneumoniae* isolates are reduced susceptibility to β -lactams (amoxicillin/clavulanate, cefotaxime) but would still retain activity against fluoroquinolones and aminoglycosides. It is then not entirely correct to say definitely that *B. subtilis* is always more sensitive than *K. pneumoniae*, drug class, and isolate variable sensitivity, and empirical treatment should be guided by local susceptibility data.

Recommendations

1. Healthcare providers should only prescribe antibiotics after proper identification and susceptibility testing. Routine surveillance checks should be conducted regularly by healthcare centers in order to monitor the resistance trend of antibiotics.
2. The use of broad-spectrum antibiotics should be restricted to confirmed infections because indiscriminate use may lead to resistant development of antibiotic resistance, and lastly, future studies should be conducted to broaden the scope by involving molecular characterization of resistance genes in *K. pneumoniae* and *B. subtilis*.

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