Antimicrobial Resistance of *Klebsiella Pnuemoniae* and *Escherichia Coli* Isolated From Tablets without Primary Packaging in Some Urban Areas of Nasarawa State, Nigeria

N. B. Okpalaeke[1]., Y. B Ngwai [1*]., I. H Nkene[1]., J. Danjuma[1]., G. A. Imokhe[1]., O. F. Bolarinwa[2]., S. C. Tama[1]., I. N. Igbawua[1]

[1]Department of Microbiology, Nasarawa State University, P.M.B. 1022, Keffi, Nigeria

[2]Federal University, Lokoja, Nigeria

***Corresponding Author**

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ABSTRACT

Microbial contamination of non-sterile pharmaceutical dosage forms is a well-documented global problem especially in poor resource countries. This study investigates the antimicrobial resistance profile of *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia coli* (*E. coli*) isolated from tablets without primary packaging in some urban areas of Nasarawa State, Nigeria. A total of 325 unsealed non-sterile solid dosage pharmaceuticals comprising of Metronidazole, Vitamin C, Paracetamol and Vitamin B complex collected were collected from Akwanga and Keffi metropolis, Nasarawa State, Nigeria. *Klebsiella pneumoniae* and *E. coli* were isolated and identified from the tablets using standard microbiological methods. The antimicrobial susceptibility of the isolates was evaluated carried out using disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) method. The overall percentage proportion of *K. pneumoniae* and *E. coli* out 325 tablets without primary packaged were 29(8.9%) and 12(3.7%). The *K. pneumoniae* and *E. coli* isolated from the tablets without primary packaging in the study area were highly resistant to amoxicillin-clavulanic acid, cephalexin, ceftriaxone and cefotaxime with percentage resistance ranges from 50% to 100.0% respectively. Hundred percent (100.0%) of the isolates from tablets were extensive drug resistant. Most unsealed non-sterile solid dosage pharmaceuticals in the study area were predominantly contaminated with *K. pneumoniae* and penicillin and cephalosporins were not effective against the Enterobacteriaceae isolated from the tablets. Stricter quality control measures, as well as public awareness and expanded surveillance will help mitigate the risks of drug-resistant infections as a result of contaminated medications.

Keywords: Pharmaceuticals, Drugs, Infection, *Klebsiella pneumoniae*, Contamination, *Escherichia coli*, Tablets,

INTRODUCTION

Non-sterile dosage pharmaceutical products, including both prescription and over-the-counter drugs, must meet stringent quality standards to ensure their safety and efficacy [1]. The presence of Gram-negative and Gram-positive bacteria, as well as fungi, in these products can compromise quality and pose significant health risks to consumers [1, 2]. Microbial contamination may occur at any stage of manufacturing, distribution, or dispensing [3]. In informal settings such as street markets, where drugs are often sold unsealed, the risk of contamination is particularly high due to inadequate storage conditions and lack of quality control measures [1].

Recent studies by [4] and [5] highlight that intact packaging serves as a critical barrier in preserving drug quality. However, a substantial proportion of medications sold in resource-limited settings are either unsealed or have damaged packaging, increasing the likelihood of contamination. The unregulated sale of such drugs by informal vendors has become a widespread concern, exposing consumers to potential health hazards associated with microbial-contaminated pharmaceuticals [1]. Unsealed drugs may be compromised through environmental

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exposure, improper storage, or even intentional adulteration [1]. These factors not only reduce therapeutic efficacy but may also introduce pathogenic microorganisms, leading to infections and adverse health outcomes.

Microbial contamination of non-sterile dosage pharmaceutical products is a well-documented global public health concern [1]. Improper storage or handling can facilitate the proliferation of pathogenic Gram-negative and Gram-positive bacteria, as well as fungi, compromising product safety [6]. Ingestion or topical application of contaminated non-sterile pharmaceuticals may lead to adverse health effects, particularly in vulnerable populations such as children, the elderly, and immunocompromised individuals [7]. Recent studies identify *Pseudomonas aeruginosa*, *Staphylococcus* spp., *Klebsiella pneumoniae*, and *Escherichia coli* as predominant microbial contaminants in these products [1, 2].

Despite growing evidence of microbial contamination, the antimicrobial resistance (AMR) profiles of such isolates remain understudied. This study therefore focuses on characterizing the AMR patterns of *Enterobacteriaceae* isolated from unpackaged tablets sold in urban areas of Nasarawa State, Nigeria.

MATERIALS AND METHODS

Study Area

This study focused on the three agro ecological zones of Nasarawa State, Nigeria. The state comprises 13 Local Government Areas (LGAs), spanning 28,735 km² [8] with an estimated population of 1,826,883 [9]. Geographically, Nasarawa State lies within latitudes 7°45'–9°45' N and longitudes 7°–10°37' E in Nigeria's North Central region. It shares borders with Taraba and Plateau States to the east, Kaduna State to the north, Benue and Kogi States to the south, and the Federal Capital Territory to the west.

The terrain consists of undulating plains averaging 400 m above sea level, interspersed with inselbergs. The climate is tropical wet-and-dry, with an annual mean temperature of 29.39°C (slightly below Nigeria's national average) and average precipitation of 136.71 mm distributed across fewer than 155 rainy days per year [10].

Inclusion and exclusion criteria

The inclusion criteria for this study consist of unsealed tablets sold by informal (untrained) drug vendors in Akwanga and Keffi metropolitan areas, Nasarawa State, Nigeria. Exclusion criteria included both sealed and unsealed tablets sold by licensed (trained) drug vendors in the same regions.

Sample size determination

The sample size for this study was calculated using the formula:

$n = Z^2 \times P\ (1-P)/d^2$ where n is the required sample size, $Z = 1.96$, $Z = 1.96$ (for a 95% confidence level), $P = 0.1044$ (10.44% prevalence based on a previous study [11], and $d = 0.05$ (precision level). Substituting these values:

$n = 1.96^2 \times 0.1044(1-0.1044)/ (0.05)^2$

$= 144$

To account for potential non-response, 125.4% proportion was added to the minimum sample size obtained 144+ (144 x 1.254) = 144 + 180.576 = 324.576

≈ 325

The sample size was increased to 325 to improve statistical power, precision, and reliability, while ensuring a robust and diverse sample.

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Sample Collection

A total of 325 unsealed tablets - including Metronidazole, Vitamin C, Paracetamol, and Vitamin B complex preparations - were aseptically collected from vendor counters in semi-urban areas of Nasarawa State. Samples were obtained using sterile plastic containers to maintain integrity. Following collection, all samples were immediately transported under appropriate conditions to the Microbiology Laboratory, Department of Microbiology, Nasarawa State University, Keffi, for microbial analysis.

Isolation of *Escherichia coli* and *Klebsiella* species

Each tablet sample was pulverized using sterile techniques, and 1.0 g of the resulting powder was suspended in 10 ml of Tryptic Soy Broth (TSB). The suspensions were incubated at 37°C for 24 hours to enrich bacterial growth. Following incubation, cultures were streaked onto MacConkey Agar (MCA) plates and incubated at 37°C for an additional 24 hours. Distinct colonies exhibiting deep or light pink coloration on MCA were subsequently subcultured onto Eosin Methylene Blue (EMB) agar plates. Presumptive identification was based on colony morphology: *Escherichia coli* was indicated by greenish colonies with a metallic sheen, while *Klebsiella* species were identified by characteristic pinkish, mucoid colonies.

Commercial Biochemical Kit (KB003 H125^{TM}) Identification of *Escherichia coli* and *Klebsiella* species

The presumptive *E. coli* and *Klebsiella* species that were Gram negative, rod shape were confirmed using KB003 H125^{TM} Kit following the manufacturer's instruction as follows. Following purification, 2 pure colonies of suspected isolates from NA plate were transfer to 5 ml of sterile normal saline in a tube to prepare a suspension and the turbidity of the suspension was adjusted to the turbidity equivalent to the turbidity of 0.5 McFarland standard.

The kit was aseptically open by sealing off the sealing foil and 50 µl of the adjusted suspension , which was inoculated into each well of the kit and it was sealed back using the sealing foil and incubated at 37ºC for 24 h. After incubation, 3 drops of reagent R036 and 1 drop of reagent R015 were added to well No 5; 2 drops of reagent R009 were added to well No. 6; 3 drops of reagent R029 and 1 drop of reagent R030 were added to well No. 9; 1 drop of reagent 1007 was added to well No. 10 and finally 1 drop of reagent R008 was added to well No. 11. The results were read and interpreted as per the standard given in the identification index.

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing of the bacterial isolates was carried out as earlier described by Clinical and Laboratory Standards Institute [12]. Briefly, three (3) pure colonies of the isolates were inoculated into 5 ml sterile 0.85% (w/v) NaCl (normal saline) and the turbidity of the bacteria suspension was adjusted to the turbidity equivalent to 0.5 McFarland's standard. The McFarland's standard was prepared as follows: 0.5 ml of 1.172% (w/v) $BaCl_2.2H_2O$ was added into 99.5 ml of 1% (w/v) H_2SO_4.

A sterile swab stick was soaked in standardized bacteria suspension and streaked on Mueller-Hinton agar plates and the antibiotic discs were aseptically placed at the center of the plates and allowed to stand for 1 h for pre-diffusion. The plates were incubated at 37ºC for 24 h. The diameter zone of inhibition in millimeter was measured and the result was interpreted in accordance with the susceptibility break point earlier described by Clinical and Laboratory Standards Institute [12].

Classification of Antibiotic Resistance

Antibiotic resistance in the isolates were classified into multidrug resistance (MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories); extensive drug resistance (XDR: non-susceptible to ≥1 agent in all but ≤2 antimicrobial categories); pan drug resistance (PDR: non-susceptible to all antimicrobial listed) [13].

RESULTS, DISCUSSION AND CONCLUSION

Overall Occurrence of *Escherichia coli* and *Klebsiella pneumoniae*

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Escherichia coli and *K. pneumoniae* were isolated from unpackaged paracetamol, vitamin B complex, vitamin C, and metronidazole tablets obtained from vendors in Akwanga and Keffi, Nasarawa State, Nigeria. Among the 325 analyzed samples, *K. pneumoniae* showed higher prevalence (29 isolates, 8.9%) compared to *E. coli* (12 isolates, 3.7%).

The distribution patterns revealed significant variations among drug types:

K. pneumoniae contamination was highest in paracetamol (25.7%, 18/70 samples) and metronidazole (7.6%, 7/92), but minimal in vitamin C (1.1%, 1/91), while *E. coli* was exclusively detected in paracetamol samples (17.1%, 12/70), with no isolates recovered from metronidazole, vitamin B complex, or vitamin C tablets

These findings are visually summarized in Figure 1.

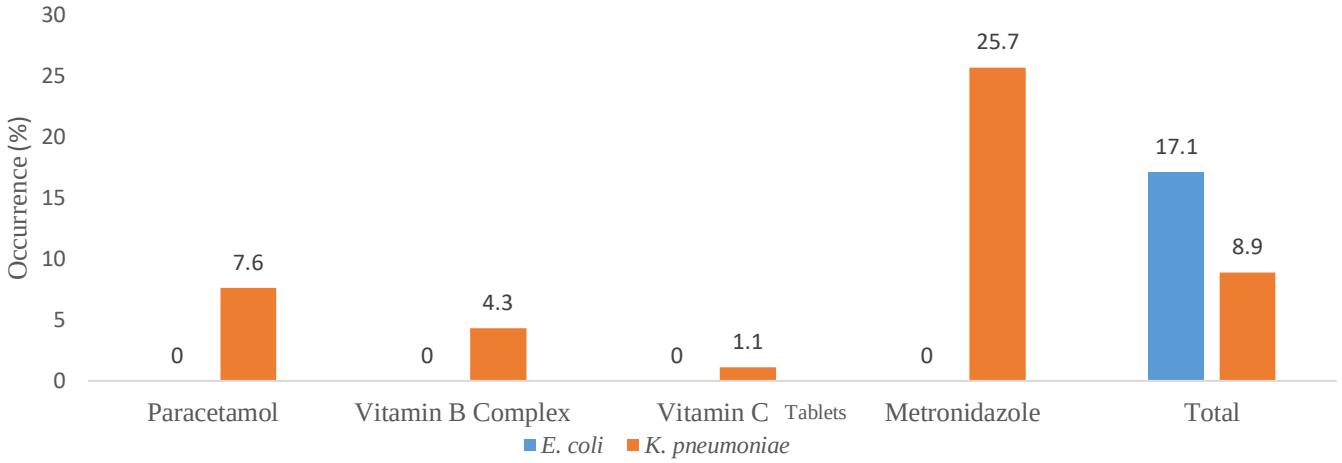

Figure 1: Occurrence of *Enterobacteriaceae* from Tablets without primary packaging in Keffi and Akwanga Metropolis, Nasarawa State, Nigeria

Occurrence of the bacteria in relation to the Study Area

The prevalence of *K. pneumoniae* and *E. coli* contamination varied significantly between study locations (Figures 2 and 3). In Keffi, *K. pneumoniae* demonstrated higher contamination rates (12.1%, 14/116 samples) compared to *E. coli* (5.2%, 6/116). Contamination patterns by drug type in Keffi revealed that for Paracetamol: 38.1% (8/21) positive for *K. pneumoniae*, while for Vitamin C: 2.5% (1/40) positive for *K. pneumoniae*. For Vitamin B complex: No *K. pneumoniae* isolates were detected

In Akwanga, *K. pneumoniae* similarly showed greater prevalence (7.2%, 15/209) than *E. coli* (2.9%, 6/209). The drug-specific contamination profile in Akwanga differed: Paracetamol: 20.4% (10/49) contamination rate and Vitamin B complex: 3.4% (2/58) contamination rate

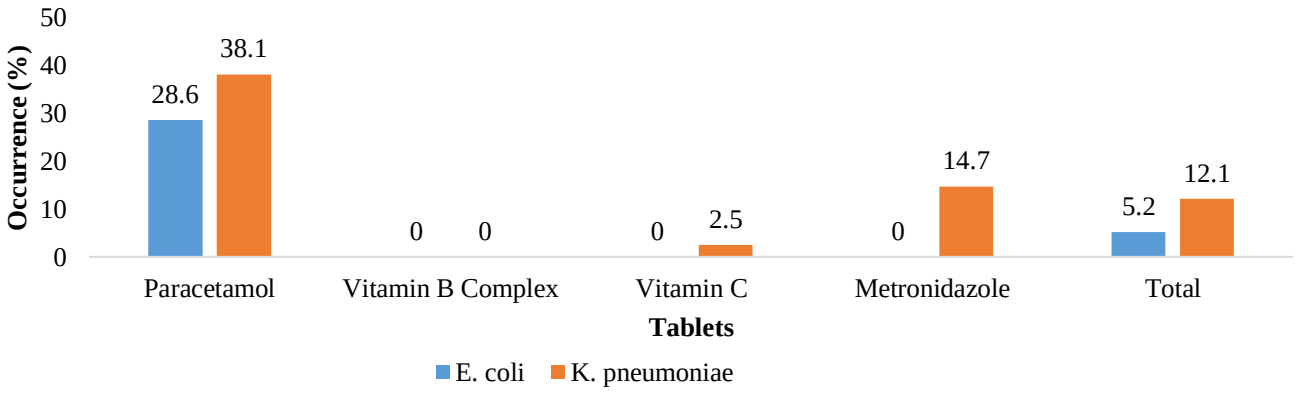

Figure 2: Occurrence of *Enterobacteriaceae* bacteria from Tablets without primary packaging in Keffi Metropolis, Nasarawa State, Nigeria

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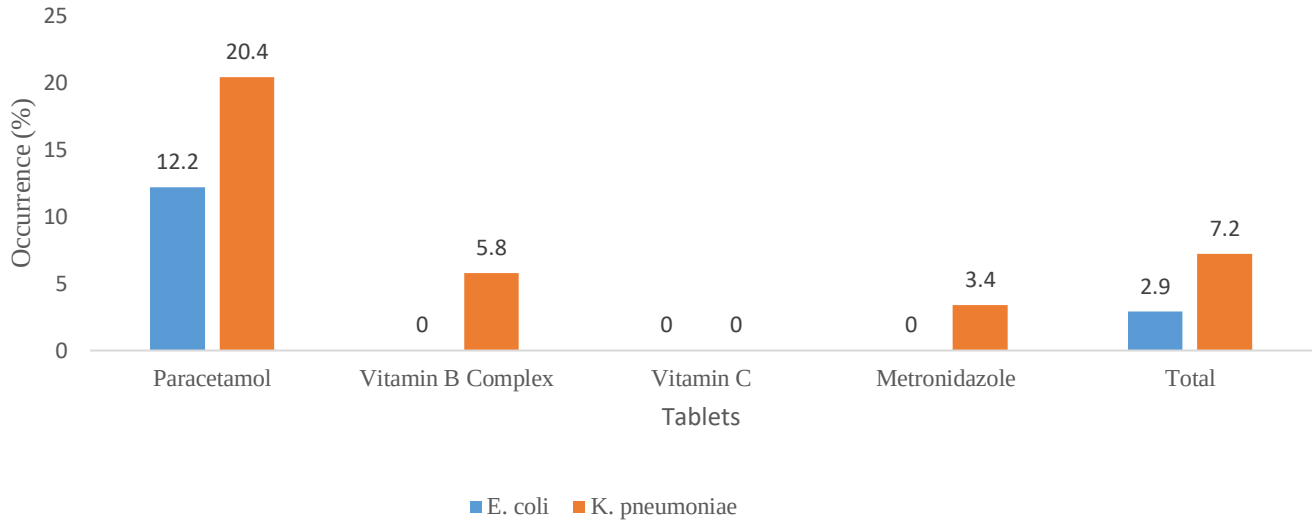

Figure 3: Occurrence of *Enterobacteriaceae* from Tablets without primary packaging in Akwanga Metropolis, Nasarawa State, Nigeria

Antimicrobial Resistance

The resistance patterns of *K. pneumoniae* and *E. coli* isolates from unpackaged tablets are presented in Figure 4. Both pathogens exhibited high resistance rates (50-100%) to Cephalexin, Amoxicillin-clavulanic acid, Cefotaxime and Ceftriaxone.

Notably, all isolates remained susceptible to Ciprofloxacin, Ofloxacin, Pefloxacin, Gentamicin, Nitrofurantoin and Streptomycin.

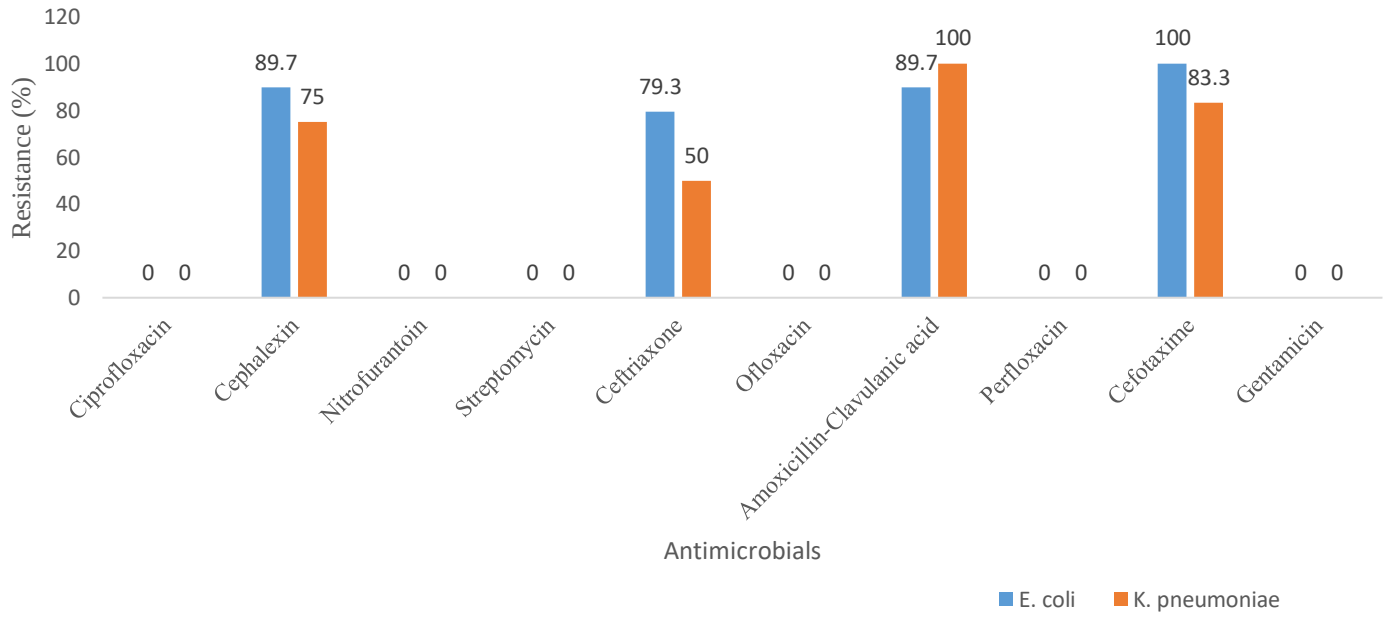

Figure 4: Antimicrobial Resistance of Enterobacteriaceae isolated from Tablets without primary packaging in Akwanga and Keffi Metropolis, Nasarawa State, Nigeria

Classification of Antimicrobial Resistance

The antimicrobial resistant *K. pneumoniae* and *E. coli* isolated from tablets in the study area were classified into MDR, XDR and PDR as shown in Table 1. All the isolates from tablets without primary packaged in the study area were XDR isolates with 100.0% occurrence.

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Table 1: Classes of antimicrobial resistance in *Klebsiella pneumoniae* and *Escherichia coli* isolated from tablets without primary packaged in Akwanga and Keffi metropolis, Nasarawa State, Nigeria

Classes of Antimicrobial Resistance	Frequency (%)	
	K. pneumoniae	*Escherichia coli*
MDR	0 (0.0)	0 (0.0)
XDR	29 (100.0)	12 (100.0)
PDR	0 (0.0)	0 (0.0)

MDR=Multidrug resistance; XDR=Extensive drug resistance; PDR=Pandrug resistance

DISCUSSION

Microbial contamination of pharmaceuticals poses significant risks, including physicochemical degradation and medicine-related infections [2]. Our findings demonstrate that unpackaged tablets in the study area were contaminated with Enterobacteriaceae, particularly *K. pneumoniae* (8.9%) and *E. coli* (3.7%). This aligns with previous reports identifying these organisms as common contaminants of non-sterile pharmaceutical products [14, 15], though contrasts with findings by [16] who reported *S. aureus* and *P. aeruginosa* as predominant species.

Notably, paracetamol tablets showed the highest contamination rates for both organisms, while E. coli was absent in vitamin C, vitamin B complex, and metronidazole samples. The observed predominance of *K. pneumoniae* may reflect several factors such as: increased environmental persistence compared to *E. coli*, enhanced survival in pharmaceutical excipients and greater resistance to desiccation. While the exact contamination sources remain undetermined, we hypothesize human contact during handling or dispensing as probable routes. This is particularly concerning for unpackaged products, which despite being manufactured in controlled environments, remain vulnerable to post-production contamination.

The detection of these opportunistic pathogens in medications raises public health concerns, as both *K. pneumoniae* and *E. coli* are established agents of healthcare-associated and community-acquired infections. Their presence in oral medications may represent a potential route for antimicrobial resistance dissemination, infection risks for immunocompromised patients and compromised therapeutic efficacy.

Our study revealed concerning resistance profiles among the isolated *K. pneumoniae* and *E. coli* strains. These organisms exhibited high resistance rates (>90%) to amoxicillin-clavulanic acid and cephalosporins (cephalexin, ceftriaxone, cefotaxime). This resistance pattern suggests limited therapeutic utility of these β-lactam antibiotics for infections caused by these particular strains, which are known pathogens in both hospital and community settings (e.g., urinary tract, bloodstream, and respiratory infections). While the precise resistance mechanisms were not investigated, the observed pattern may reflect overuse/misuse of these antibiotics in clinical and community settings, possible production of extended-spectrum β-lactamases (ESBLs), and chromosomal or plasmid-mediated resistance mechanisms.

Notably, all isolates met the criteria for extensive drug resistance (XDR) as defined by [13], demonstrating resistance to ≥1 agent in ≥2 antimicrobial categories. This finding is particularly alarming given that contaminated medications may serve as reservoirs for XDR pathogens, and these organisms were predominantly found in commonly used medications (paracetamol and vitamin B complex). In contrast, the isolates remained susceptible to Fluoroquinolones (ciprofloxacin, pefloxacin, ofloxacin), Aminoglycosides (streptomycin, gentamicin) and Nitrofurantoin. This susceptibility profile suggests these antimicrobials may retain clinical utility against these strains. The observed pattern likely reflects lower selective pressure from these antimicrobial classes, different resistance mechanisms compared to β-lactams and potential preservation of their efficacy in empirical treatment. These findings align with previous reports of ≥90% resistance to amoxicillin-clavulanic

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acid and third-generation cephalosporins [2], highlighting a persistent public health challenge. The contamination of unpackaged medications with XDR Enterobacteriaceae underscores the need for improved pharmaceutical quality control, antimicrobial stewardship programs and enhanced packaging regulations as well as surveillance of non-sterile pharmaceutical products.

CONCLUSION

This study highlights a critical public health risk in Nasarawa State, Nigeria, where unpackaged non-sterile pharmaceuticals—particularly paracetamol—were contaminated with extensively drug-resistant (XDR) *Klebsiella pneumoniae* and *Escherichia coli*. These isolates exhibited alarming resistance (50–100%) to essential antibiotics like β-lactams (cephalosporins, amoxicillin-clavulanate), suggesting compromised treatment options for common infections. The findings underscore the urgent need for stricter pharmaceutical regulations (e.g., enforced primary packaging, vendor compliance), antimicrobial stewardship (e.g., restricted antibiotic sales, AMR surveillance), and community interventions (e.g., public awareness, vendor training) to curb AMR spread. Multisector collaboration is vital to mitigate risks posed by contaminated medications, especially in informal drug markets.

Regulatory agencies must prioritize quality control for non-sterile drugs, while healthcare providers should advocate for rational antibiotic use. Community engagement programs could reduce reliance on unpackaged medications, and further research should explore contamination sources and resistance mechanisms. Addressing this issue demands integrated efforts to safeguard medication quality and combat antimicrobial resistance in low-resource settings.

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