

Multidrug-Resistant Bacteria in Public Boreholes and Vendor-Supplied Drinking Water in a University Town: A Public Health Concern.

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Abstract

Multidrug-resistant (MDR) bacteria pose a global public health threat due to their ability to withstand multiple antibiotics. This study investigated MDR bacteria in public boreholes and vendor-supplied drinking water and assessed their antimicrobial susceptibility. Water samples were cultured using the spread plate method and identified based on morphological and biochemical characteristics. Antibiotic susceptibility testing was performed using the disc diffusion method, and resistance variation across 14 antibiotics was analysed using the Chi-square (χ^2) test of independence, under the assumptions of categorical data, independence of observations, and expected cell counts ≥ 5 where applicable. All isolates exhibited multidrug resistance. *Escherichia coli* showed the highest resistance (85.7%), followed by *Klebsiella pneumoniae* (78.5%), *Staphylococcus aureus* and *Pseudomonas aeruginosa* (64.2%), and others (57.1%). A statistically significant association was observed between antibiotic type and resistance rate, $\chi^2(13, N = 41) = 362.72, p < 0.001$. Amoxicillin, Ampicillin, and Nalidixic Acid showed 100% resistance, while Tarivid and Perfloracin remained effective. These findings highlight the need for enhanced water safety and targeted antimicrobial surveillance to limit resistance transmission.

Keywords: Antibiotics; antimicrobial susceptibility; drinking water; multidrug-resistant bacteria; public health.

Introduction

Water is vital for survival, used for drinking, cooking, and sanitation. However, contaminated water can pose serious health risks, including waterborne diseases [Suggestion: pose serious health risks such as waterborne diseases] caused by bacteria. In many developing countries, including Nigeria, public boreholes are common sources of drinking water, but they are often contaminated with multidrug-resistant (MDR) bacteria, posing significant public health risks.

Studies have shown high prevalence of MDR bacteria in Nigeria's public water sources (Oluyeye et al., 2009; Odonkor & Addo 2018)

Water sold by vendors is a crucial source of drinking water, particularly in urban areas where piped water is not readily accessible (Ahmad, 2017). However, the quality of vendor-supplied water can be unpredictable, and it may contain harmful bacteria, including those resistant to multiple antibiotics (Udoh et al., 2021). The risk of contamination is higher when water is transported and stored in unclean conditions, such as in reused plastic containers that are not adequately cleaned (Imarhiagbe et al., 2023; Agbasi et al., 2024).

Bacteria are key pathogens that cause waterborne diseases, leading to widespread gastrointestinal outbreaks. Each year, nearly 1.7 billion people suffer from diarrhoea, which is the second leading cause of death among children under five, resulting in about 760,000 child fatalities annually (Manetu et al., 2021; Ali et al., 2025).

Health problems in rural areas of developing countries are largely due to the lack of access to safe drinking water (Wolf et al., 2023). Enteric bacteria from human and animal waste often contaminate water sources through runoff, which is compounded by inadequate wastewater treatment systems, leading to the discharge of pollutants (Singh et al., 2022; Wu et al., 2024; Owhonka et al., 2021).

Multidrug-resistant (MDR) bacteria are strains that are resistant to multiple antibiotics, posing a significant public health concern. Their resistance complicates treatment, leading to prolonged illnesses, higher healthcare costs, and increased mortality rates. Contributing factors include the overuse and misuse of antibiotics, poor sanitation and hygiene, and inadequate infection control measures (Bharadwaj et al., 2022).

The misuse and overuse of antibiotics in medicine and agriculture have contributed to the emergence of antibiotic-resistant bacteria. When these multidrug-resistant (MDR) strains contaminate water sources, they can be transmitted to humans, leading to challenging waterborne infections (Muteeb et al., 2023).

Contaminated water can transmit bacterial pathogens like *Escherichia coli*, *Salmonella* serovars, *Shigella*, *Vibrio cholerae*, and *Clostridium*. Additionally, pathogens that can survive in soil and water include *Legionella*, *Burkholderia pseudomallei*, and atypical mycobacteria (Kristanti et al., 2022; Wittler et al., 2023; Asif et al., 2024).

Escherichia coli has been widely used as an indicator organism for faecal contamination in water since 1895. While many strains are harmless, pathogenic variants such as *E. coli* O157:H7 can cause severe and sometimes fatal infections (Murei et al., 2024; Sorensen et al., 2021; Nowicki et al., 2021). This study aims to isolate and identify multidrug-resistant bacteria from public boreholes and vendor-supplied water in Amassoma, a university town in Bayelsa State, Nigeria.

Materials and Methods

Research Design, Study Area, and Sampling Procedure

The study adopted a descriptive cross-sectional survey design to assess the prevalence of multidrug-resistant bacteria in public boreholes and vendor-supplied drinking water. This design enabled the collection of data at a single point in time, providing a snapshot of antimicrobial resistance patterns in the study area.

Study Area

The study was conducted in Amassoma, a university town in Bayelsa State, Nigeria, focusing on both public boreholes and water vendors commonly used by the local population.

Sampling Procedure

A systematic random sampling technique was employed to select boreholes and water vendors within the study area. Sampling points were first stratified by location to ensure geographic coverage, after which every *n*th source was selected from the list of identified water points (Howell et al., 2020). This approach minimised selection bias and ensured that the collected water samples were representative of the typical water sources used by the community.

Collection of water samples

Water samples were collected using aseptic techniques. The tap was allowed to run for about a minute for the boreholes before the water was collected in sterile sample bottles. For water vendors, the water was poured into the sample bottles from their containers. Each water sample was uniquely identified with a label indicating the source and date of collection.

Ethical statement

This study involved only environmental water samples and did not include human participants; therefore, formal institutional ethics approval was not required. Permission was obtained from the Amassoma Community Development Committee, and verbal consent was secured from all participating water vendors and borehole owners.

Sample size determination

To determine the sample size for this study, we first estimated the total population of public boreholes and water vendors within the study area. The Taro Yamane formula was applied at a 95% confidence level to calculate the sample size: $n = N / (1 + N(e)^2)$. Where: *n* = required sample size, *N* = estimated population size, and *e* = margin of error (typically between 0.01 and 0.05) ((Yamane, 1967; Farzand, n.d.)

Sample processing

Water samples were processed using the spread plate technique to isolate bacteria for antimicrobial susceptibility testing. CLED and blood agar were used to support a range of bacterial growth. A 0.5 ml aliquot of each sample was evenly spread on agar plates using a sterile bent glass rod and incubated at 37°C for 18–24 hours. Distinct colonies were subcultured and identified based on colony morphology, haemolysis, Gram reaction, catalase, motility, triple sugar iron, citrate utilisation, and oxidase tests, following standard procedures described by Cheesbrough 2015). Confirmed isolates were then subjected to antibiotic susceptibility testing using the disc diffusion method.

Antibacterial susceptibility testing

Antibiotic susceptibility testing was carried out using the Kirby Bauer method. The isolates were grown overnight on nutrient agar and then suspended in sterile peptone broth using a sterile wire loop until the turbidity was equal to 0.5 McFarland standards. Sterile, non-toxic cotton swabs dipped into the standardised inoculum were used to streak the entire surface of Mueller-Hinton

agar plates. The following antibiotics were used because they are easily available over the counter and can be used with or without prescription: Augmentin (Au), Amoxicillin (Am), Ampicillin (An), Ceporex (Cep), Preflacin (Pef), Tarivid (Ofx), Ciprofloxacin (Cpx), Sparfloxacin (Sp), Pefloxacin (Pef), Nalidixic acid (Na), Gentamicin (Cn), Streptomycin (S), Chloramphenicol (CH), and Ceprin (Sxt). Antibiotic disks were carefully placed on the plates using sterile forceps. The plates were then incubated at 37°C for 24 hours. The results were analysed using CLSI guidelines.

Result

As shown in Figure 1, 25 of the 50 water samples were taken from boreholes, and the remaining 25 were collected from water vendors.

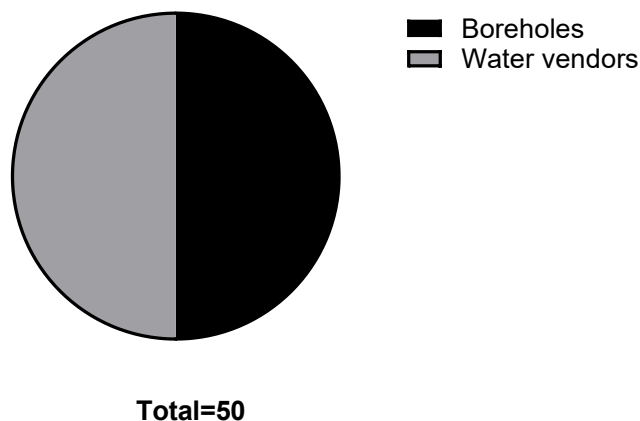


Figure 1: Distribution of water samples

Table 1: Distribution of bacterial isolates obtained from the water samples.

Bacteria	Boreholes	Water vendors	Total	Percentage
<i>S. aureus</i>	2	3	5	12.19%
Coagulase-ve <i>Staphylococcus</i>	1	-	1	2.44%
<i>Streptococcus</i> spp	1	3	4	9.76%
<i>E. coli</i>	4	7	11	26.83%
<i>P aeruginosa</i>	2	5	7	17.07%
<i>K pneumonia</i>	5	4	9	21.95%
<i>Proteus</i> spp	2	2	4	9.76%
Total	17	24	41	100%

Table 1 shows that bacterial isolates are more prevalent in vendor-supplied water (58.54%) than in boreholes (41.46%). *E. coli* was the most common species (26.83%), followed by *Klebsiella pneumoniae* (21.95%) and *Pseudomonas aeruginosa* (17.07%), all of which are linked to waterborne diseases and antibiotic resistance. Other species included *Staphylococcus aureus* (12.19%), *Proteus* spp. (9.76%), and *Streptococcus* spp. (9.76%). The high presence of pathogens,

particularly in vendor-supplied water, underlines significant public health risks and underscores the need for improved water safety measures.

Table 2a, Table 2b and Table 2c summarises the antibiotic susceptibility profiles of *Staphylococcus aureus* (n = 5), coagulase-negative *Staphylococcus* (CoNS) (n = 1), and *Streptococcus pneumoniae* (n = 4) against 14 antimicrobial agents. *S. aureus* showed 100% sensitivity to ofloxacin (OFX), rifampicin (REF), and penicillin (PN), but was fully resistant to ampicillin (AM) and nalidixic acid (NA). Cefuroxime (CEP) and Augmentin (AU) demonstrated moderate activity, while ciprofloxacin (CPX) yielded mixed responses. The CoNS isolate was generally sensitive to most antibiotics but exhibited resistance to AM, NA, trimethoprim-sulfamethoxazole (SXT), chloramphenicol (CH), and gentamicin (CN), indicating potential multidrug resistance. All *S. pneumoniae* isolates were susceptible to OFX, REF, PN, and pefloxacin (PEF), while showing high resistance to AM, NA, streptomycin (SP), SXT, and CH. Intermediate susceptibility was observed with CEP and CPX.

These findings suggest that newer-generation antimicrobials, particularly fluoroquinolones and rifampicin, remain effective against the tested Gram-positive organisms. In contrast, older and commonly used antibiotics such as AM and NA showed limited efficacy, underscoring the need for local antimicrobial stewardship and susceptibility-guided therapy.

Table 2a: Antibiotic susceptibility pattern of *Staphylococcus aureus* (5)

	S P	A M	N A	SX T	OF X	C H	RE F	P N	PE F	A U	CE P	CP X	S
Sensitive	-	-	-	1	5	5	5	5	2	4	1	-	-
Resistance	4	5	5	4	-	5	5	3	2	1	4	-	-
Intermediate	1	-	-	-	-	-	-	2	1	-	-	-	-

Table 2b: Antibiotic susceptibility pattern of Coagulase-negative *Staphylococcus* (1)

	S P	A M	N A	SX T	OF X	C H	RE F	P N	PE F	A U	CE P	CP X	C S	C N
Sensitive	-	-	-	1	1	1	1	1	1	1	1	-	-	1
Resistance	1	1	1	1	1	1	1	1	1	1	1	-	-	1
Intermediate	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2c: Antibiotic susceptibility pattern of *Streptococcus pneumoniae* (4)

	S P	A M	N A	SX T	OF X	C H	RE F	P N	PE F	A U	CE P	CP X	C S	C N
Sensitive	-	-	-	2	4	4	4	2	1	3	2	3	-	-

Resistance	4	4	4	3	4	4	4	2	2	2	1	-	-	-
Intermediate	-	-	-	-	-	-	-	1	1	-	-	-	-	-

Table 3a, Table 3b and Table 3c summarise the antibiotic susceptibility patterns of Gram-negative bacteria: *Proteus* spp. (n = 4), *Klebsiella pneumoniae* (n = 9), and *Escherichia coli* (n = 11). Isolates were tested against 13 antibiotics and classified as sensitive, resistant, or intermediate.

Proteus spp. demonstrated 100% sensitivity to ofloxacin (OFX), chloramphenicol (CH), rifampicin (REF), and penicillin (PN). Moderate sensitivity was observed to trimethoprim-sulfamethoxazole (SXT), pefloxacin (PEF), Augmentin (AU), and ciprofloxacin (CPX). Resistance was highest to ampicillin (AM), nalidixic acid (NA), and CH.

Klebsiella pneumoniae exhibited considerable resistance overall, with 100% sensitivity only to SXT and REF. Partial sensitivity was observed for CPX, PN, and CH, while complete resistance was recorded for AM, NA, and streptomycin (SP).

Escherichia coli demonstrated extensive multidrug resistance, with 100% resistance to AM, REF, and PN, and high resistance to CH, NA, and SXT. However, full sensitivity was recorded for OFX and PEF. Moderate susceptibility was observed for AU, cefuroxime (CEP), CPX, and streptomycin (S).

These results shows significant antimicrobial resistance among the Gram-negative isolates, particularly *E. coli* and *K. pneumoniae*. The superior activity of fluoroquinolones such as OFX and PEF suggests their potential utility as empiric treatment options in regions with similar resistance profiles.

Table 3a: Antibiotic susceptibility pattern of *Proteus* spp (4)

	SP	AM	NA	SXT	OFX	CH	REF	PN	PEF	AU	CEP	CPX
Sensitive	1	-	-	2	4	4	4	4	-	2	1	3
Resistance	2	4	4	2	-	4	4	-	1	2	1	-
Intermediate	1	-	-	-	-	-	-	1	-	-	-	1

Table 3b: Antibiotic susceptibility pattern of *Klebsiella pneumonia* (9)

	S	A	N	SX	OF	C	RE	P	PE	A	CE	CP	C
	P	M	A	T	X	H	F	N	F	U	P	X	S
Sensitive	2	9	-	9	5	-	9	4	3	9	7	7	7
Resistance	7	9	9	7	9	3	9	5	4	5	4	2	1
Intermediate	2	-	-	2	-	-	-	-	-	2	-	-	1

Table 3c: Antibiotic susceptibility pattern of *Escherichia coli* (11)

	S P	A M	N A	SX T	OF X	C H	RE F	P N	PE F	A U	CE P	CP X	C S	C N
Sensitive	1	-	-	11	1	11	11	2	4	8	7	7	7	7
Resistance	9	11	11	8	10	11	8	6	3	4	1	-	1	1
Intermediate	1	-	-	2	-	-	-	1	-	1	-	3	-	-

Table 4: Variation in resistance rates across antibiotics

Antibiotics	Resistant	Intermediate	Susceptible
Augmentin (AU)	16(60%)	6(14.63%)	10(24.39%)
Amoxicillin (AM)	41(100%)	-	-
Ampicilin (PN)	41(100%)	-	-
Ceporex (CEP)	22(53.66%)	6(14.63%)	13(31.70%)
Reflacine(PEF)	3(7.31%)	-	38(92.67%)
Tarivid (OFX)	-	-	41(100%)
Ciprofloxacin (CPX)	7(17%)	5(12.20%)	29(70.73%)
Sparfloxacin (SP)	31(75.6%)	6(14.63%)	4(9.76%)
Perfloxacin (PEF)	-	-	41(100%)
Nalidixic Acid (NA)	41(100%)	-	-
Gentamycin (CN)	7(17.07%)	4(9.76%)	30(73.17%)
Streptomycin (S)	-	-	41(100%)
Chloramphenicol(CH)	38(92.68%)	-	3(7.31%)
Septtrin(SXT)	10(24.39%)	3(7.31%)	28(68.29%)

Table 4 summarises the overall resistance, intermediate, and susceptibility patterns observed across 14 commonly used antibiotics. To determine whether resistance patterns varied significantly by antibiotic type, a Chi-square test of independence was conducted. The test revealed a statistically significant association between antibiotic type and resistance rate, $\chi^2(13, N = 41) =$

362.72, $p < 0.001$, indicating that resistance was not uniformly distributed across the antibiotics tested.

High resistance levels were observed for amoxicillin (AM), ampicillin (PN), and nalidixic acid (NA), each showing 100% resistance among isolates. Chloramphenicol (CH) and sparfloxacin (SP) also exhibited elevated resistance rates of 92.68% and 75.6%, respectively. In contrast, perflocacin (PEF), tarivid (OFX), and reflacine (PEF2) demonstrated complete or near-complete susceptibility, suggesting varying effectiveness among antibiotic classes.

These findings reinforce the presence of multidrug-resistant bacteria in community water sources, highlighting the urgent need for targeted antimicrobial surveillance, evidence-based prescribing, and local resistance monitoring programs.

Discussion

The World Health Organization (WHO) recognises antimicrobial resistance (AMR) as a global health emergency and a central pillar of the One Health approach, which integrates human, animal, and environmental health (WHO,2023; WHO,2024). One of the key mechanisms driving AMR is horizontal gene transfer (HGT), through which antimicrobial resistance genes (ARGs) are disseminated via mobile genetic elements, such as plasmids and transposons. Increasing evidence indicates the presence of antimicrobial-resistant bacteria (ARB) in drinking water systems worldwide, highlighting the dual role of water as both a reservoir and a transmission pathway for resistance genes (Cristina, 2021).

Although the WHO water quality guidelines prioritise the reduction of faecal contamination, they currently do not address the risks associated with antimicrobial resistance genes (ARGs) in drinking water. This omission is significant, as the environmental dissemination of ARGs poses a growing threat to public health by potentially compromising the effectiveness of critical clinical antimicrobials (Sobsey et al., 2014).

In this study, bacterial isolates were recovered from both public boreholes and vendor-supplied water. The distribution revealed a higher prevalence in vendor-supplied water (58.54%) compared to borehole water (41.46%), indicating greater vulnerability associated with informal water handling and storage systems. *Escherichia coli* was the most frequently isolated organism (26.83%), indicating faecal contamination and poor sanitary conditions. Other prevalent organisms included *Klebsiella pneumoniae* (21.95%) and *Pseudomonas aeruginosa* (17.07%), both of which are commonly associated with healthcare-related infections and exhibit intrinsic resistance mechanisms.

Less frequently isolated species included *Staphylococcus aureus* (12.19%), *Proteus* spp. (9.76%), and *Streptococcus* spp. (9.76%). Coagulase-negative *Staphylococcus* (2.44%) was also detected, highlighting the microbial diversity present in these water sources.

The detection of *P. aeruginosa* suggests possible environmental contamination and biofilm formation within storage containers or distribution systems. These findings underscore the importance of implementing improved water handling practices, conducting regular microbiological monitoring, and providing public health education to mitigate the risk of infection and environmental exposure to resistant bacteria. The organisms identified are associated with a range of infections, including gastrointestinal, urinary tract, and respiratory illnesses, particularly

in vulnerable populations such as children and immunocompromised individuals (Cristina et al., 2021; Aljamali et al., 2021; Duhaniuc et al., 2024).

Contamination of drinking water with resistant bacteria is often associated with anthropogenic activities, including the misuse of antibiotics in agriculture, veterinary medicine, and human healthcare. Runoff from farms and effluent from wastewater treatment plants introduce both antibiotics and resistant microorganisms into surface and groundwater sources (Vaz-Moreira et al., 2021; Neher et al., 2020).

Our findings are consistent with previous studies in Nigeria and other African countries (Popoola et al., 2024), which reported a high prevalence of multidrug-resistant bacteria, including *Pseudomonas*, *Staphylococcus*, and *Streptococcus*, in various water sources. In another study (Adegoke, 2022) isolated similar species from borehole water within a university environment, with 100% resistance observed in *Pseudomonas*, *Streptococcus*, *Staphylococcus*, *E. coli*, and *Klebsiella*. Notably, our study did not detect *Salmonella* spp., which Adegoke reported. Similarly, (Mohamed et al., 2021) found high rates of MDR *E. coli*, *Pseudomonas*, and *Klebsiella* in bottled water in Kenya. In contrast, a systematic review by Rabiou et al. found relatively lower multiple antibiotic resistance (MAR) indices (0.04–0.1) in *E. coli* isolates from African drinking water, suggesting geographical variation in the intensity of resistance.

Our study revealed alarmingly high resistance levels across several commonly used antibiotics. Resistance was universal (100%) for Amoxicillin (AM), Ampicillin (PN), and Nalidixic Acid (NA), rendering them ineffective against the tested bacterial population. Chloramphenicol (CH) and Sparfloxacin (SP) also showed high resistance rates of 92.68% and 75.6%, respectively. These findings suggest widespread resistance to first-line and even some second-line antibiotics, likely due to unregulated antibiotic use and environmental exposure.

While some antibiotics showed moderate effectiveness, such as Ciprofloxacin (CPX, 70.73% susceptible) and Gentamycin (CN, 73.17% susceptible), their associated resistance rates (17% and 17.07%, respectively) remain concerning. Encouragingly, Tarivid (OFX), Perfloxacin (PEF), and Reflaxine (PEF2) exhibited 100% and 92.7% susceptibility, indicating they may still be viable options for treating waterborne infections. However, overreliance on these agents may lead to eventual resistance, emphasising the need for careful stewardship.

Statistical analysis confirmed that the variation in resistance rates across antibiotics was significant ($\chi^2 = 362.72$, $p < 0.001$), indicating that not all antibiotics are equally effective against waterborne bacteria. This finding reinforces the importance of localised surveillance and susceptibility testing to guide treatment and public health interventions.

The presence of multidrug-resistant bacteria in community water sources, such as boreholes and vendor-supplied drinking water, poses a significant threat to public health in low- and middle-income countries, including Nigeria (Otokunefor & Otokunefor, 2021; Gandra et al., 2020; Ramatla et al., 2023), and waterborne AMR pathogens can undermine health systems by causing infections that are difficult and costly to treat (Coque et al., 2023).

The detection of multidrug-resistant bacteria in public boreholes and vendor-supplied water has significant public health implications. Water is a basic necessity for life, and when contaminated with pathogenic microorganisms, especially those resistant to multiple antibiotics, it becomes a vehicle for disease transmission and an amplifier of antimicrobial resistance in the community.

Contaminated water sources can expose large segments of the population—including vulnerable groups such as children, the elderly, and immunocompromised individuals—to infections that are increasingly difficult to treat. Ingestion or contact with multidrug-resistant (MDR) pathogens may result in waterborne diseases such as diarrhoea, urinary tract infections, and wound infections that fail to respond to standard first-line antibiotics. This can lead to prolonged illness, higher healthcare costs, and increased risk of mortality.

Moreover, the indiscriminate use of antibiotics, particularly in informal or unregulated settings, often in response to recurrent infections, may further exacerbate resistance. Poor water quality thereby creates a vicious cycle: frequent infections lead to increased antibiotic use, which in turn selects for and promotes the proliferation of resistant bacterial strains.

Conclusion

The study's findings reveal significant resistance to antibiotics like amoxicillin and ampicillin, underscoring the urgent need for public health interventions. Key measures include enhancing water treatment and sanitation, regulating water vendors, conducting regular water quality assessments, and promoting antimicrobial stewardship and education on hygiene and antibiotic use. Without these actions, multidrug-resistant (MDR) bacteria in community water sources will pose a serious threat to public health and hinder progress in combating infectious diseases and antimicrobial resistance (AMR).

Limitations

This study provides baseline data on MDR bacteria in drinking water from Amassoma, but its findings may not be generalizable. It lacked molecular methods to detect specific resistance genes and focused only on culturable organisms, potentially missing viable but non-culturable pathogens.

Despite these limitations, the research is significant for AMR surveillance in university communities with informal access to water. The integration of susceptibility testing with statistical analysis enhances the findings and supports future studies using broader methods and geographic areas.

Acknowledgement

The authors express their sincere gratitude to the CDC Chairman of Amassoma Town for his support and cooperation, as well as to the water vendors who participated in this study.

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