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EVALUATION OF COMMON ANTIMICROBIALS AGAINST CLINICAL ISOLATES: AN IN VITRO STUDY

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ABSTRACT

Purpose: *In this era of COVID-19, one of the most effective protective measures to prevent respiratory diseases is maintaining hand hygiene. Moreover, the use of hand sanitizer, hand wash, antiseptics, and disinfectants increased abruptly in this outbreak.*

Materials & Methods: *An in vitro antimicrobial potential of twelve antimicrobials were chosen for this study. They were tested for their antibacterial activity using disk diffusion and agar well diffusion methods against eleven clinical isolates from urine, wounds, tracheal aspirate, and sputum.*

Results: *Clinical isolates were* presumptively identified using standard microbiological procedures as *Escherichia coli*, *Klebsiella* sp., *Staphylococcus aureus*, *Acinetobacter* sp., and *Streptococcus* sp. Among all the antimicrobials, Savlon (family size) antiseptic disinfectant showed the highest zone of inhibition (ZOIs) against most of the bacterial isolates, followed by *hand sanitizer Hexisol. Whereas, the least antimicrobial activity was observed by Savlon hand wash, Germ Kill hand sanitizer, and Dabur hand sanitizer among all the tested products.*

Conclusions: *The variability in performance of those twelve products against bacterial pathogens revealed an urge to validate the antibacterial activity of antimicrobials and systematic monitoring of their effectiveness and uniformity in activity against pathogenic microbes.*

INTRODUCTION

Hospitals, which are a vital source of multidrug resistant pathogens and community acquired infections, are escalating and thus pose a serious public health problem worldwide (Otokunefor & Princewill, 2018; Ramzi *et al*., 2020). Because hands are thought to be the primary route for transmitting microbes and infections to people (Ramzi *et al*., 2020; Chojnacki *et al*., 2021), the density and species of pathogens that colonize people's hands are highly variable and can be influenced by a variety of factors such as age, gender, ethnicity, and profession (Ramzi *et al*., 2020), the density and species of pathogens that colonize the hands of individuals are highly variable and can be influenced by a number of factors, including age, sex, ethnicity, and profession (Chojnacki *et al*., 2021).

According to the Centers for Disease Control and Prevention (CDC), the World Health Organization (WHO), and many other health experts, hand hygiene by either hand washing or sanitizing is well known as one of the most significant activities essential for the reduction of microbial burden, microbial transmission, hospital-acquired infections, reduced gastrointestinal and respiratory illness, and improved overall health (Ramzi *et al*., 2020; Chojnacki *et al*., 2021; Jain *et al*., 2016; Ochwoto *et al*., 2017).

The emergence of the COVID-19 (Coronavirus Disease 2019) pandemic has led to the extensive use of hand disinfectants (Jing *et al*., 2020). Hand disinfectants are commercially available in various types and forms, such as anti-microbial soaps, water-based or alcohol-based hand sanitizers. Different types of delivery systems are also formulated, for instance, rubs, foams, or wipes (Jing *et al*., 2020). However, disinfection is achieved by antimicrobial agents such as synthetic disinfectants (quaternary ammonium compounds, QAC), povidone-iodine, triclosan, alcohols, ethanol, isopropanol, or propanol, halogenated compounds such as sodium hypochlorite, peroxygen compounds such as hydrogen peroxide, and aldehydes such as glutaraldehyde (Otokunefor & Princewill, 2018; Jain *et al*., 2016; Ochwoto *et al*., 2017). Alcohol-based sanitizers comprised of either 80% ethanol or 75% isopropyl alcohol are more potent, but concentrations higher than 80% alcohol are less potent because proteins are not easily denatured in the absence of water (Gold *et al*., 2022). The prompt and active antimicrobial potency against broad spectrum of bacteria and viruses was proven by alcohol-based hand sanitizer (ABHS) that was recommended by World Health Organisation (WHO). However, ABHS use against non-enveloped viruses is uncertain due to limited research (Jing *et al*., 2020).

In response to the SARS-CoV-2 pandemic, there has been increased recognition of the importance of hand hygiene, which has led to an overwhelming increase in hand sanitizer demand, though they have minimal activity against bacterial spores, protozoan oocytes, and some non-enveloped (non-lipophilic) viruses (Ramzi *et al*., 2020; Chojnacki *et al*., 2021; Ochwoto *et al*., 2017; Jing *et al*., 2020). In fact, a few challenges and concerns with regard to this formulation in terms of fire hazards and skin toxicity due to high alcohol content [Jing *et al*., 2020] as well as most of these products have made numerous claims of reducing "germs and harmful bacteria" by 99.9%. Some studies have observed an apparent increase in the concentration of bacteria in handprints impressed on agar plates after cleansing. Hence, there still exists a need for verification of these claims by the regulatory authorities for the enforcement of good quality measures (Ramzi *et al*., 2020; Jain *et al*., 2016; Ochwoto *et al*., 2017). From these perspectives, the current pandemic and corresponding demand for hand rubs encourage this *in vitro* comparative study to assess the antimicrobial activity of hand sanitizers with a few other antimicrobials against clinical isolates.

MATERIALS AND METHOD

Test products:

Twelve antimicrobials were randomly selected in this study. Among them, eleven were purchased on March 11, 2022, from nearby pharmacy shops in the Siddeshwari area of Dhaka, Bangladesh, and one was a 70% alcohol that was commonly used for laboratory purposes. A photograph of those antimicrobials such as hand sanitizers (9), hand wash (1), antiseptic disinfectant (1), and cleaning reagent (1) were presented in Figure 1, and their properties were presented in Table 1.

Figure 1: A photograph of test products in Bangladesh that were used in the study.

Table 1: Test products used in the study and their properties

Product No.	Test products	Type	Viscosity	Ingredients
1	Germ kill	Hand sanitizer	viscous	70% isopropyl alcohol, among others.
2	Dr. Rhazes	Hand sanitizer	viscous	70% ethanol, lemon extracts and proprietary anti-microbial compounds
3	Dabur	Hand sanitizer	viscous	Ethanol (66% w/w) and others.
4	Mr. hygine	Hand sanitizer	watery	Water, Ethanol, Triethyl amine, Trichlosen, Sepimex zen, Sodium methylparaben, Perfume, etc.
5	Savlon	Hand sanitizer	viscous	Ethyl alcohol denatured, aqua, isopropyl alcohol, propylene glycol, fragrance, glycerine, disodium EDTA, sodium hydroxide, aloe leaf extracts, iodophenyl acetate, linalool, citronellol, etc.
6	Hexisol	Hand sanitizer	watery	Chlorhexidine Gluconate 0.5% w/v in 70% v/v Isopropyl alcohol.
7	Dettol	Hand sanitizer	viscous	Alcohol denatured, water, propylene glycol, tetrahydroxypropyl ethylenediamine, fragrance, limonene, etc.
8	Sepnil	Hand sanitizer	viscous	Ethanol, Carbomer, Glycerin, Polyethylene Glycol, TEA, Aqua, and Perfume.
9	Lifebuoy	Hand sanitizer	viscous	Water, glycerine, carbomer copolymer, camellia sinensis leaf extrat, aloe barbadensis leaf extract, d-menthol, triethanolamine, ethyl alcohol 70%. (Ethyl Alcohol, Isopropyl Alcohol, Niacinamide)
10	Savlon (family size)	Antiseptic disinfectant	watery	Chlorhexidine Gluconate solution BP 0.3% w/v and strong Cetrimide Solution BP 3% w/v, Cetostearyl Alcohol, Liquid Paraffin, Methylhydroxybenzoate (E218), Propylhydroxybenzoate (E216), Disodium Edetate, Perfume & Purified Water
11	Savlon (handwash)	Hand wash	viscous	Sodium Laureth Sulfate, Cocamidropropyl Betaine, Cocodi Ethanol Amide, Benzophenon 3, Sorbitol, Citric Acid, Sodium Choride, Perfume, Preservative, CI 42090, CI 60730, Aqua.
12	70% ethanol	Cleaning reagent (laboratory use)	watery	95% ethanol and water.

Source: Authors

Test microorganisms

The bacterial isolates used in this study for the evaluation of antimicrobial activities of test products were previously stocked at -20°C in the laboratory of the Department of Microbiology, Stamford University Bangladesh. A total of 10 clinical bacterial isolates, including *E. coli* (5 isolates), *Klebsiella* sp. (2 isolates), *Staphylococcus aureus*, *Acinetobacter* sp., and *Streptococcus* sp., were collected from urine, wounds, tracheal aspirates, and sputum. Bacteria were subcultured on nutrient agar media (HiMedia Laboratories, Mumbai, India). Their morphology was rechecked by microscopy, colony morphology, and biochemical tests before being tested for their susceptibility to test products in the current study.

Antimicrobial susceptibility by the well diffusion method:

The agar well diffusion test was carried out to assess the antimicrobial activities of tested antimicrobials such as hand sanitizers. This involved the use of an inoculum corresponding to the 0.5 McFarland standard (approximately 10^8 CFU/ml). The test inoculum was swabbed onto a Mueller Hinton Agar (MHA) plate and allowed to stand at room temperature for 15 minutes. Following this, 5 wells were created on the plates using a sterilized 6 mm cork borer and 50 µl of the test substances were added into each of the four wells, while the fifth one was incorporated with sterile water (Control). After 24 hours of incubation at 37°C, the ZOIs were measured. Each test was performed in triplicate and the average of all readings was taken as the ZOI in each case.

Antimicrobial susceptibility by disk diffusion method:

Antimicrobial activity of hand sanitizers and other test substances was obtained using the disc diffusion method. In short, the clinical bacterial suspensions previously adjusted with the 0.5 McFarland standards (approximately 10^8 CFU/ml) were inoculated onto respective petridishes containing MHA media. Sterilized filter paper discs (6 mm in diameter) were placed on the surface of each medium and were impregnated with 25 ml of each test substance. After 24 hours of incubation at 37°C, the diameters of the ZOIs were measured in mm. Each test was performed in triplicate and the average of all readings was taken as the ZOI in each case.

An antibiotic susceptibility test of the isolates

The antibiotic sensitivity of test microorganisms was performed on Mueller-Hinton agar according to the Kirby Bauer disc diffusion (agar-disc-diffusion) method. The antibiotic discs used in this experiment were amoxicillin, amikacin, amoxiclav, ampicillin, azithromycin, cefixime, ceftazidime, ceftriaxone, cefuroxime, cephradine, ciprofloxacin, colistin, doxycycline, gentamycin, imipenem, metropenem, nalidixic acid, nitrofurantion, cotrimoxazole, vancomycin, tazobactam, linezolid, dotripenum, tigecycline, clindamycin, levofloxacin, and cepepime. The plates were then incubated at 37°C for 24 hours. After incubation, the plates were examined, and the zones of inhibition (ZOIs) were measured in mm according to the standard guidelines (Bauer *et al*., 1966; Banik *et al*., 2018).

RESULTS AND DISCUSSION

A total of 12 antimicrobials, including 9 hand sanitizers, 1 hand wash, 1 antiseptic disinfectant, and 1 cleaning reagent (laboratory use), were comprised in our study. All of them were alcohol-based and mostly contained ethanol and water with additional ingredients.

The clinical isolates were rechecked for their microscopic (Table 2), colony morphology (Table 3), and biochemical features (Table 4). Among 10 clinical isolates, most of them were gram negative bacteria like *E. coli* (05), *Klebsiella* sp. (02), and *Acinetobactor* (01) with two gram positive bacteria such as *Staphyococcus aureus* (01), and *Streptococcus* sp. (01) were identified from different clinical samples (Table 2, 3, and 4).

Table 2: Microscopic observation of the tested isolates

Serial No.	Lab strain designation	Origin	Shape	Arrangement	Gram reaction
1	T-37612	Urine	rod	Single	Negative
2	M-815	Urine	rod	Single	Negative
3	TC-3163	Wound	Spherical	Clusters	Positive
4	223	Urine	rod	Single	Negative
5	T-37808	Urine	rod	Single	Negative
6	153	Tracheal aspirate	Rod	Small, cluster	Negative
7	M-976	Sputum	Cocci	Chain	Positive
8	T-38459	Urine	Rod	Single	Negative
9	M-922	Wound	Rod	Single	Negative
10	TC-3262	Urine	Rod	Single	Negative

Source: Authors

Table 3: Cultural properties of the tested isolates

Serial No.	Lab strain designation	Origin	Media	Size	Pigmentation	Form	Margin	Elevation
1	T-37612	Urine	MAC	Large	pink	Irregular	Entire	Crateriform
2	M-815	Urine	MAC	Large	pink	Circular	Entire	Convex
3	TC-3163	Wound	BA	Moderate	white	Circular	Undulate	Raised
4	223	Urine	MAC	Large	pink	Circular	Entire	Raised
5	T-37808	Urine	MAC	Moderate	pink	Circular	Entire	Convex
6	153	Tracheal aspirate	MAC	small	pink	Circular	Entire	Raised
7	M-976	Sputum	BA	small	white	Circular	Entire	Raised
8	T-38459	Urine	MAC	Moderate	pink	Irregular	Undulate	Crateriform
9	M-922	Wound	MAC	small	pink	Irregular	Undulate	Crateriform
10	TC-3262	Urine	MAC	Moderate	pink	Irregular	Undulate	Crateriform

Note: MAC=MacConkey agar, BA=Blood agar.
Source: Authors

Table 4: Biochemical tests of the tested isolates

Serial No.	Lab strain designation	MR	VP	TSI				Indole	Citrate	Presumptive Identification
				Slant	Butt	H_2S	Gas			
1	T-37612	+	-	A	A	-	+	+	-	*E. coli*
2	M-815	+	-	A	A	-	+	-	+	*Klebsiella* sp.
3	TC-3163	+	-	A	A	-	-	-	+	*S. aureus*
4	223	+	-	K	K	-	-	+	+	*Klebsiella* sp.
5	T-37808	+	-	A	A	-	+	+	-	*E. coli*
6	153	+	-	A	K	-	-	-	-	*Acinetobacter* sp.
7	M-976	+	-	K	K	-	-	-	-	*Streptococcus* sp.
8	T-38459	+	-	A	A	-	-	+	-	*E. coli*
9	M-922	+	-	K	K	-	-	+	-	*E. coli*
10	TC-3262	+	-	K	K	-	-	+	-	*E. coli*

Note: MR=Methyl Red, VP=Voges-Proskauer, TSI=Triple Sugar Iron, K=Alkaline, A=Acidic.
Source: Authors

According to the data in Table 5, comparing all the products in the disk diffusion and agar well diffusion methods, the range of ZOIs of all bacteria was elevated in the case of products no. 6 (Hexisol, hand sanitizer) and no. 10 (Savlon, antiseptic disinfectant), except for *Streptococcus* sp. The latter showed wide ZOI against products no. 2 (Dr. Rhazes, hand sanitizer) and no. 6 (Hexisol, hand sanitizer). Almost all the tested bacteria showed limited ZOIs, for some it was 0 mm, towards products no. 1 (Germ Kill hand sanitizer), 3 (Dabur hand sanitizer), and 11 (Savlon hand wash). The ZOIs were higher in the agar well diffusion method than in the disk diffusion method. Although twice the volume of antimicrobials were used in the agar well diffusion method, the ZOIs were not proportionally increased.

Table 5: Zones of inhibition (ZOIs) of 12 antimicrobials in mm against ten clinical isolates were determined using disk and agar well diffusion methods.

Serial No.	Clinical isolate	Lab strain designation	Tests	Test product number and their ZOIs (in mm)											
				1	2	3	4	5	6	7	8	9	10	11	12
1	*E. coli*	T-37612	Disk diffusion	0	0	8	8	8	15	7	7	8	40	0	8
			Agar well diffusion	0	0	14	13	10	22	10	12	11	27	0	22
2	*Klebsiella* sp.	M-815	Disk diffusion	0	9	9	0	12	14	8	8	7	16	0	8
			Agar well diffusion	0	12	10	0	14	21	10	10	12	30	0	20
3	*S. aureus*	TC-3163	Disk diffusion	0	12	0	7	10	16	0	0	7	17	10	6
			Agar well diffusion	0	16	0	11	12	22	0	0	12	30	14	11
4	*Klebsiella* sp.	223	Disk diffusion	8	10	8	12	10	15	9	8	10	20	10	11
			Agar well diffusion	11	11	11	12	12	22	10	10	12	25	11	12
5	*E. coli*	T-37808	Disk diffusion	7	10	7	8	7	16	7	0	11	16	0	7
			Agar well diffusion	10	13	9	14	10	31	14	0	13	32	0	16
6	*Acinetobacter* sp.	153	Disk diffusion	7	10	9	0	9	12	10	8	9	16	0	9
			Agar well diffusion	10	10	10	0	10	22	15	10	11	27	0	18
7	*Streptococcus* sp.	M-976	Disk diffusion	7	17	0	0	0	20	8	7	10	0	0	7
			Agar well diffusion	10	22	0	0	0	32	10	10	10	0	0	8
8	*E. coli*	T-38459	Disk diffusion	10	7	10	12	14	14	8	7	9	15	8	7
			Agar well diffusion	11	16	10	13	15	24	10	10	13	31	10	11
9	*E. coli*	M-922	Disk diffusion	7	7	0	7	10	12	12	7	7	15	0	7
			Agar well diffusion	10	13	0	12	13	23	10	10	12	27	0	12
10	*E. coli*	TC-3262	Disk diffusion	0	7	0	7	7	10	8	7	8	20	0	7
			Agar well diffusion	0	14	0	11	11	25	10	10	10	29	0	14

Source: Authors

Based on Table 6, the antibiotic sensitivity pattern analysis of gram-positive and gram-negative bacteria showed higher sensitivity to colistin (100%), tigecycline, and linezolid. No pathogens were found completely sensitive or resistant to all the antibiotics. However, the lowest sensitivity was observed against azithromycin, cefixime, ceftazidime, ceftriaxone, and cefuroxime. Moreover, the most resistant bacterial isolates, *E. coli* (T-37612), showed sensitivity only against colistin and tigecycline; *Klebsiella* sp. (223) against colistin, doxycycline, and tigecycline; and *Acinetobacter* sp. (153) against colistin and tigecycline.

Table 6: An antibiogram of clinical isolates

Serial No.	Clinical isolates	amoxycillin	amikacin	amoxiclav	ampicillin	azithromycin	cefixime	ceftazidime	ceftriaxone	cefuroxime	cephradine	ciprofloxacin	colistin	doxycycline	gentamycin	imipenem	metropenem	nalidixic acid	nitrofurantion	cotrimoxazole	vancomycin	tazobactam	linezolid	dotripenum	tigecycline	clindamycin	levofloxacin	cepepime
1	*E. coli* (T-37612)	-	R	R	-	R	R	R	R	R	-	R	S	R	R	R	R	-	-	R	-	R	-	R	S	-	R	R
2	*Klebsiella* (M-815)	-	S	S	-	R	R	R	R	R	-	R	S	R	S	S	S	S	S	S	-	S	-	S	I	-	R	R
3	*S. aureus* (TC-3163)	-	S	R	-	R	R	-	R	R	R	R	-	S	R	-	R	-	-	R	S	-	S	-	S	R	R	-
4	*Klebsiella* (223)	-	R	R	-	R	R	R	R	R	-	R	S	S	R	R	R	R	R	R	R	-	-	R	S	-	R	R
5	*E. coli* (T-37808)	-	S	S	I	-	S	S	S	S	-	S	S	S	S	S	S	R	S	R	-	S	-	S	S	-	S	S
6	*Acinetobacter* sp.(153)	-	R	R	-	R	R	R	R	R	-	R	S	R	R	R	R	-	-	R	-	R	-	R	S	-	R	R
7	*Streptococcus* sp. (M-976)	S	R	S	-	S	I	-	R	R	S	I	-	S	R	-	S	-	S	R	-	S	S	-	S	S	I	-
8	*E. coli* (T-38459)	-	S	S	-	R	R	S	R	R	-	S	S	I	S	S	S	S	S	S	-	S	-	S	S	-	S	S
9	*E. coli* (M-922)	-	S	R	-	R	R	R	R	R	-	S	S	R	S	S	S	S	S	S	-	I	-	S	S	-	S	R
10	*E. coli* (TC-3262)	-	S	R	-	R	R	R	R	R	-	R	S	S	S	S	R	R	S	S	-	R	-	R	S	-	R	R

Note: S=Sensitive, R=Resistant, I=Intermediate, - =Not done.

Discussion:

Infection with environmental microbes is increasing alarmingly. Normal human skin always harbors bacteria (10^2 and 10^6 CFU/cm^2) [Jain *et al*., 2016]. Thus, hand hygiene, whether hand washing or sanitizing, is well known for its importance in reducing pathogens on hands. WHO emphasizes hand hygiene in healthcare to sensitize the hands and to reduce the spread and infectivity of coronavirus. In our study, all the antimicrobials did not work evenly against all the clinical isolates. Even the highly antibiotic-resistant isolates such as *E. coli* (T-37612), *Klebsiella* sp. (223) and *Acinetobacter* sp. (153) were found highly sensitive towards Savlon antiseptic disinfectant and Hexisol hand sanitizer, followed by Dettol hand sanitizer and 70% alcohol. Most of the isolates were partially susceptible to the antimicrobials used in our study. One previous study conducted in 2016 showed that Dettol hand sanitizer was not effective against *E. coli* and *S. aureus*, which relates somewhat to our study where we found its effectiveness against *E. coli* but not *S. aureus* (Oke *et al*., 2013). Our study also corroborates with other findings where it was observed that Dettol hand sanitizer was ineffective against *Staphylococcus aureus, S. epidermidis*, and *Pseudomonas* [Tambekar *et al*., 2007; Ichor *et al*., 2018]. However, the latter finding does not agree with the findings of Kimura *et al*. (Kimura *et al*., 2004), who revealed that Dettol hand sanitizer was effective against *S. aureus* and S. *epidermidis* with ZOIs of 5 mm against each of them. It was published that two gene families of chlorohexidine-resistant genes (qacA/B and qacC/D) and fluoroquinolone efflux transporter protein (norA) [Leelaporn *et al*., 1994; Bush *et al*., 1986; Suchomel *et al*., 2013; Zmantar *et al*., 2011] in *Staphylococcus* sp. are mostly found on bacterial plasmids (qacA/B) and chromosomes (norA) [Zmantar *et al*., 2011]. Their resistance to many antimicrobial agents was due to the encoded proton-dependant export proteins and their efflux system [Ekizoğlu *et al*., 2016]. The multidrug efflux system might play a crucial role in conferring the resistance mechanism (Wakshlak *et al*., 2019).

The most effective antimicrobials found in our study were Haxisol hand sanitizer and Savlon antiseptic disinfectant against all the clinical isolates except the latter showed no inhibition zone against *Streptococcus* sp. (M-976). Most of our tested materials are alcohol-based. Though those sanitizers are made up of ethanol, isopropyl alcohols, and hydrogen peroxides in different combinations, these preparations may become toxic to human health and the environment when misused and overused. On the other hand, excessive use of alcohol-based sanitizers increases the permeability of skin, deprives oil and water from skin, and leads to skin roughness and irritation. Dry and damaged skin is a hotbed for many diseases-causing bacteria with an increased risk of virus entry into the skin (Mahmood *et al*., 2020). Although the impact of sanitizers on bacteria like antibiotic resistance is a matter of controversy, some previous studies have described the emergence of bacterial pathogens that become tolerant to alcohol-based sanitizers through unknown genetic and molecular mechanisms (Jain *et al*., 2016; Mahmood *et al*., 2020; Lobie *et al*., 2021). This tolerance could be induced by the constituents of sanitizers, such as alcohol, quaternary ammonium compounds, phenols, hydrogen peroxide, and surfactants, which cause microbial DNA damage, or benzalkonium chloride (BAC) and triclosan, which have antimicrobial properties. Not only does BAC have broad spectrum antimicrobial activity against bacteria, fungi, and viruses, but it also creates a selective environment that favors some microbial phenotypes, and thus, exposure to it can confer cross-resistance to various antimicrobial agents (Lobie et al., 2021). Due to a shortage of scientific literature, a valid comparison was unconceivable.

Traditionally, to assess the antimicrobial activity of any compound, agar well diffusion and agar dilution methods were commonly performed. Due to technique-sensitivity, alteration of properties and problems with homogenous dissolution of some tested components, the agar dilution method was not a technique of choice. Therefore, agar well diffusion and disk diffusion techniques were chosen for our study. The beneficial properties of the agar disk diffusion method are that the chemical properties of the sanitizer remain unchanged, making it easy and less technique-sensitive (Jain *et al*., 2016).

CONCLUSION

Increasing demand for hand sanitizer in the era of COVID-19 led various companies to produce hand sanitizer to cope with the need to restrict infections and transmission in the community. Thus, the variability in performance of those antimicrobials pose an urge to regularly monitor the quality to ensure international standards and uniformity in activity against pathogenic microbes and in association with that it is required to implement stringent control measures by regulatory authorities and manufacturers.

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