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Original Research Article

Molecular Characterization of Virulence Factors and Antimicrobial Resistance in Foodborne *Klebsiella Pneumoniae* Isolated from Camel Meat

Mohammed Abd Alabbas Molaghi[1*], Asseel Abdul Ridha Saeed[1]

[1]Department of Veterinary Public Health, College of Veterinary Medicine, University of Al-Qadisiyah

***Corresponding Author:** Mohammed Abd Alabbas Molaghi
Department of Veterinary Public Health, College of Veterinary Medicine, University of Al-Qadisiyah

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Abstract: Camel meat is extensively consumed across various regions" especially in the Middle East. *Klebsiella Pneumoniae* screening was performed on 100 camel meat samples from Diwaniyah, Iraq, slaughterhouses. Foodborne *Klebsiella Pneumoniae* was associated with multiple complications. The investigation revealed this bacterium in 38% of the samples"m Many isolates were tetracycline and ampicillin resistant. Testing indicated that gentamicin, ciprofloxacin, and imipenem were more effective against isolates. They tested the isolates' pathogenicity and resistance genes with PCR. The ecp A adhesin-encoding gene found in several isolates78.9%, Many isolates have the blaNDM gene26.3%, during the research and khe hemolysin gene 57.9%. Most isolates resist to Tetracycline and Trimethoprim-Sulfamethoxazole The research emphasizes food antibiotic resistance monitoring and animal processing and slaughter hygiene. Public health and infectious disease prevention rely on it. Viral and multidrug-resistant *Klebsiella Pneumoniae* in camel meat needs more research. The research stresses the need of food antibiotic resistance monitoring and meat processing and slaughter sanitation. Public health and foodborne disease prevention rely on this. More study is needed on viral and multidrug-resistant *Klebsiella Pneumoniae* in camels.

Keywords: Klebsiella Pneumonia, Camel Meat, Hemolysin Gene (khe), Antimicrobial Resistance, BlaNDM Gene, Virulence Factors, PCR Detection.

INTRODUCTION

Unsanitary meat slaughter, processing, and storage may increase the risk of foodborne disease. The WHO (2015) says this is a worldwide issue with major effects. The bacteria *Klebsiella Pneumoniae* have been found in meat, dairy, and vegetables, and research links them to food illness (Kang *et al*., 2018). In locations where cow farming is difficult, camel meat provides nutrition for folks with limited food supply. Due to its low fat and high protein content, camel meat is becoming more popular. It's a delicacy in many cultures (Kadhem *et al*., 2014). Around 35 million camels live in the Arabian Peninsula, East Africa, and South Asia, where camel meat is a staple (FAO, 2020). Culturally, camels are symbols. (FAO, 2020). Camel emblems are seen in many cultures. This is shown by their widespread consumption in Somalia, Sudan, and Saudi Arabia (Al-Mutairi, 2018). Food security is one of several cultural and economic aspects affecting camel meat. Warn that camel meat may transmit microbes. (Al-Harbi *et al.*, 2020). Meat can be infected by these germs during shipping, slaughter, environmental exposure, and handling. The Enterobacteriaceae family includes Gram-negative, facultative anaerobe *Klebsiella Pneumoniae*. It opportunistically causes pneumonia, UTIs, septicemia, and wound infections in people and animals (Budshon and Ullmann, 1998). Pathogens use capsules, cilia, siderophores, and hemolysins to kill tissues and elude the immune system. (Pachosa and Mexas, 2016). Multidrug-resistant strains of *Klebsiella Pneumoniae*, particularly those carrying carbapenemase genes such as blaNDM, pose a public health risk. Resistance also limits treatment options and increases mortality (Nordman *et al*., 2011) Studies have revealed that *Klebsiella Pneumoniae* infection in meat products varies depending on

geographic region, animal species, and hygiene practices. Detecting this bacteria in camel flesh requires constant surveillance. Multidrug-resistant strains make treatment harder, increasing illness and death worldwide. Understanding *Klebsiella Pneumoniae* pathogenicity and resistance genetics is essential for creating effective diagnostic and treatment methods. Bacteria use virulence factors to infiltrate, avoid the immune system, and destroy tissues. (Aldali 2025) Capsular polysaccharides limit phagocytosis and complement-mediated cell death, although lipopolysaccharides, cilia, and appendages aid adherence. Microbes need iron, which sephorophores help them get. Clinical diagnosis and epidemiological monitoring require rapid and accurate virulence and antibiotic resistance gene identification. PCR is a sensitive and precise way to test bacterial isolates and clinical samples for target genes. This research proposes to identify *Klebsiella Pneumoniae* genes such ecpA, blaNDM, and khe. Biofilm production and host cell adhering are caused by the common filament operon virulence factor ecpA. However, the blaNDM gene creates a New Delhi-type beta-lactamase enzyme that resistant carbapenems and other beta-lactam antibiotics. The khe gene is another genetic marker for *Klebsiella Pneumoniae*, encoding a species-specific hemolysin. This gene is conserved in *Klebsiella Pneumoniae* strains and can be distinguished from similar species. Public health concerns include the global spread of blaNDM-producing *Klebsiella Pneumoniae* via mobile genetic elements such as plasmids, highlighting the need for effective detection tools to guide surveillance and treatment.

MATERIAL AND METHOD

Sample Preparation and Isolation of *Klebsiella Pneumoniae* from Meat Samples

About (100) Camel meat samples were collected aseptically from a local slaughterhouse in Al-Diwaniyah city to investigate the presence of *Klebsiella Pneumoniae*. Approximately 25 grams of meat were aseptically placed into sterile stomacher bags for transport to the microbiology laboratory under refrigerated conditions (4°C) to preserve sample integrity (ISO 6887-3, 2017). Samples were homogenized in 225 mL of buffered peptone water to create a 1:10 dilution, followed by incubation at 37°C for 18-24 hours in buffered peptone water, then cultured on MacConkey agar and Eosin Methylene Blue (EMB) agar plates to isolate Gram-negative Enterobacteriaceae. Colonies with characteristic K. pneumoniae morphology (mucoid, lactose-fermenting pink colonies on MacConkey) were sub-cultured for purification for selective isolation of *Klebsiella Pneumoniae*, to differentiate Klebsiella species by unique colorimetric colony characteristics chromogenic medium used was HiCrome™ Klebsiella Selective Agar (HiMedia, India), (Humphries *et al.*, 2018).

Antimicrobial Susceptibility Testing

To determine the extent to which certain microbes can resist antibiotics, antibiotic susceptibility testing was performed. The Kirby-Bauer method was used to spread discs on Mueller-Hinton agar plates. After incubating the plates at a warm temperature (37°C) for 18–24 hours, the areas where microbes could not grow were measured. The results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2023). The microbes were categorized into three groups: susceptible, meaning the antibiotic was effective against them; moderately susceptible, meaning the antibiotic was somewhat effective but not highly so; and resistant, meaning the antibiotic was completely ineffective.

Testing Biofilm Phenotype (Congo red Agar Test):

The Congo red agar test can determine *Klebsiella Pneumoniae* biofilm phenotype. Congo red dye and sugar are added to heart and brain extract or tryptic soy agar to cultivate bacteria for the experiment. Incubating materials at 37°C for 24–48 hours reveals colonies. Biofilm-producing bacteria produce dry, crystalline black colonies, while non-biofilm-producing bacteria are red or pink. Congo red dye interacts with the bacteria's exterior polysaccharide matrix to change its appearance. (Naghy, *et al*., 2020). Detecting biofilm growth is important because it promotes bacterial drug resistance and the host's immunological response, prolonging infection. Sabharwal *et al*., say, biofilm formation is a complex process, and understanding its underlying mechanisms contributes to the development of new therapeutic approaches.

DNA Extraction

The QIAamp DNA Mini Kit from Qiagen (Germany) was employed to extract the DNA from the confirmed bacteria. Followed the directions on the kit, which included breaking down the bacteria, attaching the DNA to a specific column, washing it, and extracting the DNA in a solution that was liquid. Next, we used a machine called the Nanodrop 2000 from Thermo Fisher Scientific in the United States to determine the quality and quantity of DNA Measuring the absorbance of extracted DNA at 260 nm, 280 nm, and 230 nm provides insight into its quality and purity. Only tested DNA with a ratio of 1.8 to 2.0 since it indicated it was good enough. By following these steps, we can make sure that our DNA is pure and usable, which is crucial for our research.

Statistical Analysis

Was performed using the Chi-square (χ^2) test to compare the distribution of virulence and resistance genes among the *Klebsiella Pneumoniae* isolates. A p-value < 0.05 was considered statistically significans.

RESULT

Table 1: Primers used in study

Gene	Primer Sequences (5'-3')	Product Size (bp)	Reference
ecpA	F: [GGTTCACCGGGACATCATGT], R: [AGGGCCAGAAGGTGCTTTTT]	717	JN051492.1
blaNDM	F: [CAGTCGCTTCCAACGGTTTG], R: [ATCACGATCATGCTGGCCTT]	521	FN396876.1, complement region
Khe hemolysin gene	F: [TTACGTCTCAACCGGTTGGG], R: [AGCATCCGGGTAAAAAGGGG]	467	AF293352.1.

Table 2: PCR Master Mix Composition and Conditions

Component	Volume per 25 µL Reaction	Final Concentration	Manufacturer / Source
DNA Template	2 µL	Variable	Extracted from isolates
Forward Primer)	1 µL	0.4 µM	
Reverse Primer	1 µL	0.4 µM	
2X PCR Master Mix	12.5 µL	1X	Qiagen, Germany
Nuclease-Free Water	8.5 µL	—	Qiagen, Germany

Table 3: PCR Thermal Cycling Condition

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	5 minutes	1
Denaturation	95°C	30 seconds	35
Annealing	55-60°C*	30 seconds	35
Extension	72°C	1 minute	35
Final Extension	72°C	7 minutes	1
Hold	4°C	∞	—

*Annealing temperature adjusted depending on primer used (ecpA: ~58°C, blaNDM: ~57°C, khe: ~56°C).

Figure 1 and 2: show cultures isolated of *Klebsiella Pneumoniae* the colonies are identified by their distinct blue-green spots on Chromogenic agar, and their pink and mucoid appearance on MacConkey agar, which differentiates them from other bacteria based on color and lactose fermentation

Fig. 2: Biofilm formation of *Klebsiella Pneumoniae* on Congo Red Agar showing black, dry, crystalline colonies indicative of strong biofilm production."

Table 4: The distribution of virulence and resistance genes among *Klebsiella Pneumoniae* isolates

Gene	Positive	Negative	Percentage
ecpA	30	8	78.9%
blaNDM	10	28	26.3%
khe hemolysin gene	22	16	57.9%

Table 5: The correlation between antibiotic resistance and virulence genes in *Klebsiella Pneumoniae* isolates

Gene	Status	Resistant (n)	Sensitive (n)	Total (n)	χ^2	p-value
ecpA	Positive	13	17	30	4.12	0.042*
	Negative	2	6	8		
blaNDM	Positive	8	2	10	9.56	0.002**
	Negative	7	21	28		
khe	Positive	10	12	22	1.35	0.245 ns
	Negative	5	11	16		

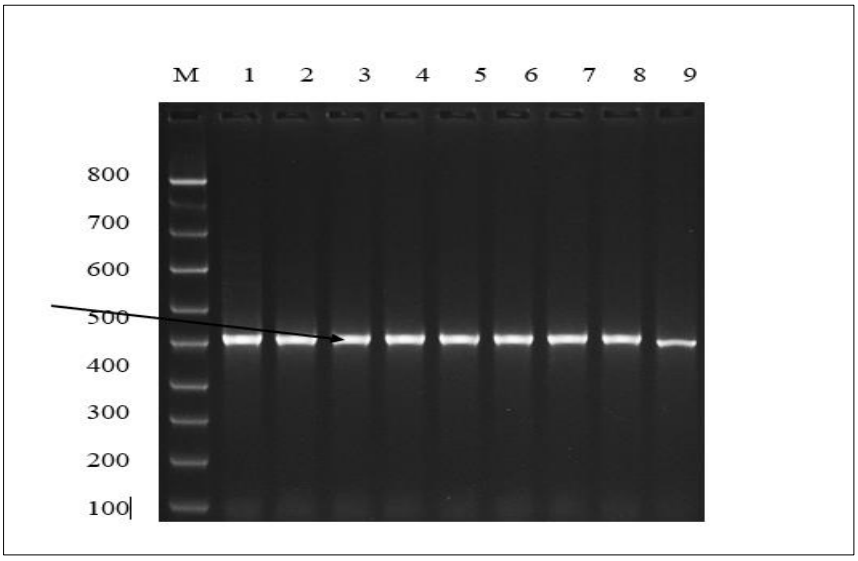

Figure 3: Agarose gel electrophoresis of *Klebsiella Pneumoniae* blaNDM gene PCR products with an expected product size of 521 bp. Lanes 1 to 10 show clear, single blaNDM gene fragment bands, indicating *Klebsiella Pneumoniae* isolate amplification. Lane M: DNA l

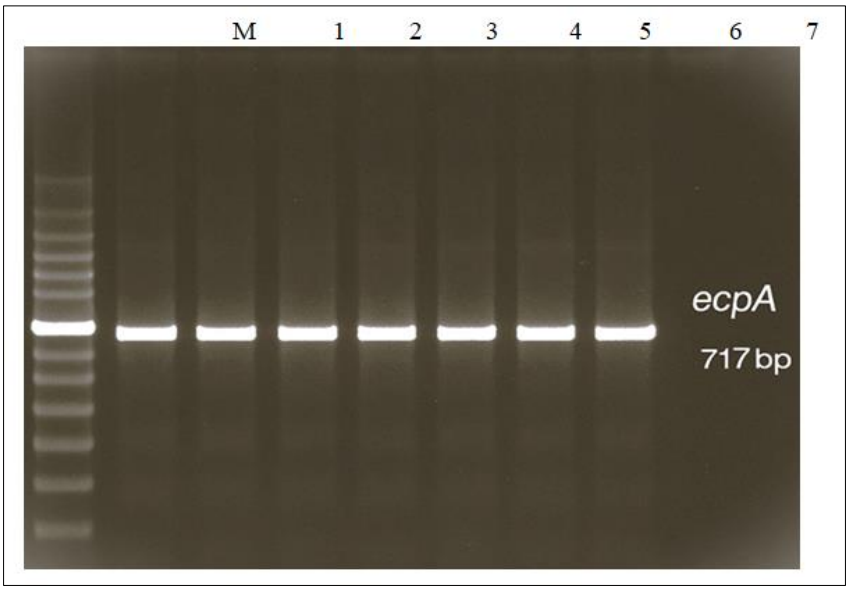

Figure 4: Agarose gel electrophoresis of *Klebsiella Pneumoniae* ecpA gene PCR products with an expected product size of 717 bp. Lanes 1o 7 show clear, single ecpA gene fragment bands, indicating *Klebsiella Pneumoniae* isolate amplification. Lane M: DNA ladder

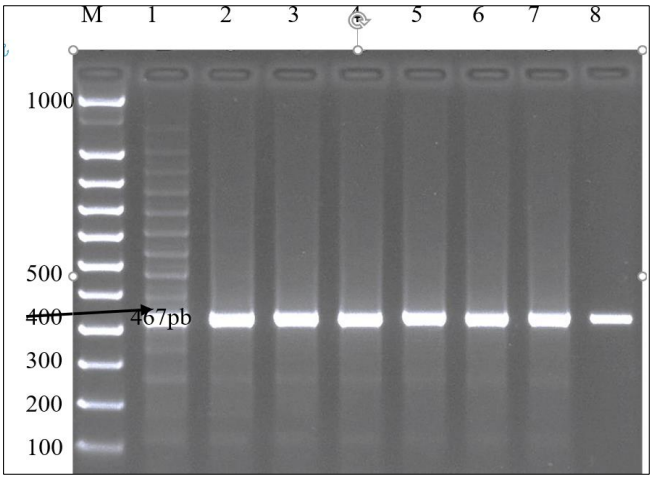

Fig. 5: Agarose gel electrophoresis of *Klebsiella Pneumoniae* Khe hemolysin gene PCR products with an expected product size of 467 bp. Lanes 1 to 8 show clear, single gene fragment bands, indicating *Klebsiella Pneumoniae* isolate amplification. Lane M: DNA ladder

Table 6: The interpretation of inhibition zone diameters was performed according to the CLSI guidelines

Antibiotic Name	Count	Range (mm)	Mean Â± SD (mm)	
Amoxicillin-Clavulanic Acid	9	14.0 - 20.0	17.11 Â± 1.90	sensitivity
Ampicillin	7	6.0 - 10.0	8.14 Â± 1.35	sensitivity
Ceftriaxone	9	13.0 - 23.0	18.67 Â± 3.28	sensitivity
Chloramphenicol	9	10.0 - 22.0	16.44 Â± 3.88	sensitivity
Ciprofloxacin	9	15.0 - 25.0	20.67 Â± 3.28	sensitivity
Gentamicin	8	14.0 - 22.0	18.38 Â± 2.67	sensitivity
Imipenem	9	18.0 - 28.0	23.33 Â± 3.04	Sensitive
Tetracycline	11	10.0 - 18.0	14.18 Â± 2.52	Resistance
Trimethoprim-Sulfamethoxazole	9	13.0 - 19.0	16.22 Â± 1.99	Resistance

Table (6). The ecpA gene was detected in 30 isolates (78.9%), representing the most prevalent gene among the tested isolates. The khe hemolysin gene was detected in 22 isolates (57.9%), while the blaNDM gene was detected in 10 isolates (26.3%). Statistical analysis using the Chi-square test revealed a significant difference in the distribution of these genes among the isolates ($\chi^2 = 17.3$, $p < 0.001$.

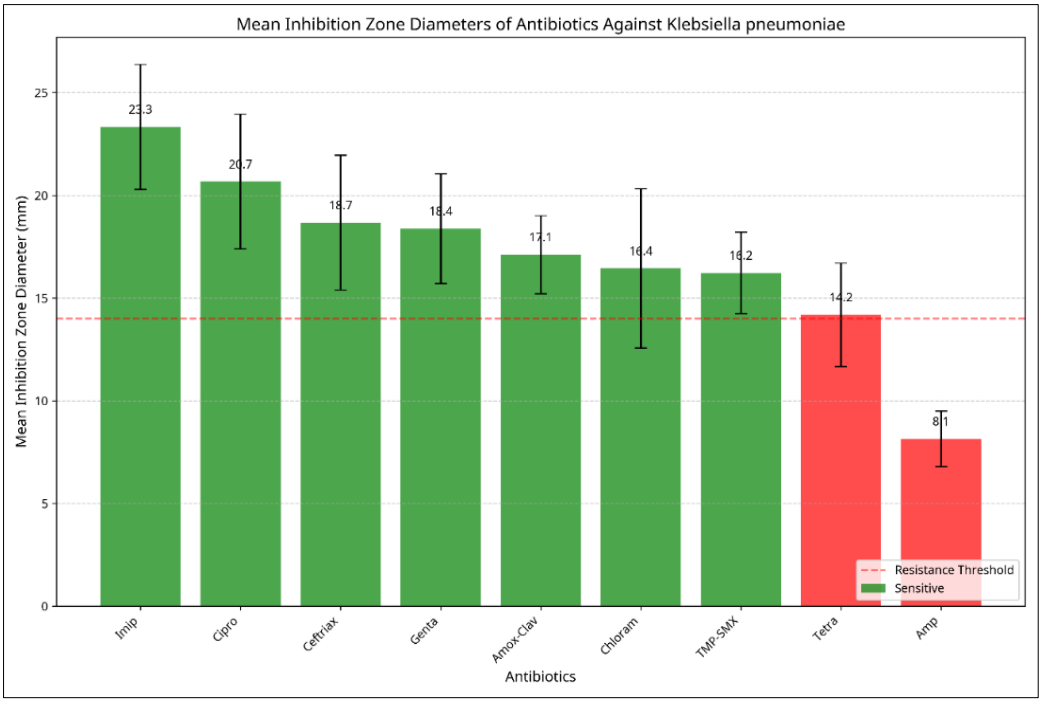

Fig. 6: Mean inhibition zone diameters for *Klebsiella Pneumoniae* isolates against different antibiotics, reflecting their susceptibility profiles

DISCUSSION

The prevalence of *Klebsiella Pneumoniae*, molecular characteristics and antibiotic resistance, transmitted by Iraqi camel meat. The study's 38% bacterium detection rate raises food safety and public health concerns because camel meat may carryas opportunistic infections. Handling, ambient air, and poor slaughterhouse hygiene cause 20%–40% contamination in Middle Eastern and other meats (Al-Harbi *et al*., 2020; Rivas, 2021). Azway *et al*., (2024) found *Klebsiella Pneumoniae* in milk and dairy products, confirming its food chain prevalence. Most EcpA isolates formed biofilms and adhered to cells .(%78.9) 57.9% of the hemolysin-producing isolates carried the khe gene. The common protrusions (ECP) of Escherichia coli, encoded by the EcpA protein, help the bacteria colonize host tissues and non-living surfaces (Deng *et al*., 2019). This gene was frequently detected in meat-borne *Klebsiella Pneumoniae* isolates, indicating resistance to washing and sterilization. This makes the bacteria more dangerous when the gene leads to host cell lysis and tissue damage (Lee *et al*., 2020). The high prevalence in this study may be due to poor slaughter and processing hygiene and cross-contamination of surfaces, water, and equipment. Manure and dirt spread *Klebsiella Pneumoniae* in animal production (Cote, 2024). The use of these drugs by veterinarians to promote growth and prevent disease may have led to selective pressure and the emergence of resistant strains, and our study found significant resistance. Epinephrine, ciprofloxacin, and gentamicin are the most effective against *Klebsiella Pneumoniae* isolates (Azway *et al*., 2024). Some isolates are resistant to last-line drugs via the blaNDM gene. Since the khe gene was not associated with antibiotic resistance, hemolysin-mediated pathogenesis is independent. This supports previous findings indicating that virulence and resistance genes arise under diverse selection conditions. Of particular concern is that 26.3% of the isolates carried the blaNDM gene. The NDM enzyme can degrade several beta-lactam antibiotics, including carbapenems. Foodborne blaNDM isolates can cause highly resistant bacteria. Food contributes to the spread of resistance genes, as clinical and food samples worldwide contain blaNDM-producing strains of *Klebsiella Pneumoniae* (Pitout *et al*., 2020). Carbapenemase production may contribute to multidrug resistance given the close association between the blaNDM gene and antibiotic resistance ($p = 0.002$). The relative probabilities of infection suggest that blaNDM isolates are more resistant. Global epidemiological statistics classify carbapenemase-producing *Klebsiella Pneumoniae* strains as the most problematic clinical and foodborne pathogens. The ecpA gene may link adhesion to antibiotic resistance ($p = 0.042$). Under antibiotic stress, biofilms form a barrier that protects bacteria from antibiotics. Reduced antibiotic diffusion and altered metabolism lead to resistance in bacteria associated with biofilms. The khe gene is not associated with resistance ($p = 0.245$), demonstrating that not all virulence factors generate antibiotic resistance. Both environment and genetics influence a bacteria's ability to cause disease and its resistance/virulence. This study suggests that foodborne antibiotic-resistant *Klebsiella Pneumoniae* poses a public health risk. The "One Health" concept links antibiotic and harmful bacteria resistance in animals, humans, and the environment (Al-Dhamir *et al*., 2025). High tetracycline and trimethoprim-sulfamethoxazole resistance is typical of foodborne bacteria worldwide. Low-income countries with limited antibiotic access have widespread *Klebsiella Pneumoniae* beta-lactam, tetracycline, and sulfonamide resistance, according to Cote (2024). Foodborne virulence and resistance isolates can infect immunocompromised patients. The findings recommend that the animal production sector reduce antibiotic use and practice hygienic slaughter and processing. Food chain resistance genes, such as blaNDM, also require continuous monitoring. The "One Health" approach should address human, animal, and environmental health to combat antibiotic resistance. Failure to address this problem could lead to an increase in treatment failures globally and the spread of multidrug-resistant bacteria.

CONCLUSION

Klebsiella Pneumoniae was identified in camel meat samples from a slaughterhouse in Al-Diwaniyah, suggesting a risk of foodborne disease. EcpA, blaNDM, and khe were found to be key virulence and resistance genes. These genes were also shown to cause pathogenicity and antibiotic resistance. The observed resistance to tetracycline and ampicillin underscores the emergence of multidrug-resistant *K. pneumoniae* in the food chain." The strain's drug resistance proves this. This study shows that frequent monitoring and clean slaughtering can prevent antibiotic-resistant germs from spreading.

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