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Antibiotic resistance pattern of Klebsiella pneumoniae a major problem for society

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***Abstract*---**Aim: Antibiotic resistance pattern of klebsiella pneumoniae a major problem for society. Methods: After ethical approval from the institutional ethical committee this study was done in the department of microbiology from April 2021 to march 2022 in genesis institute of dental science and research centre with collaboration of anil baghi hospital, firozpur, Punjab India. Demographic profile of all the patients like age, gender, history of any diseases was noted. All the sample like urine, sputum, blood, pleural fluid and urethral discharge were collected in the department for isolation and identification of K. pneumoniae. After 24hrs those were positive sample, further proceed for grams staining. B D Phoenix advanced automated microbiology system was used for identification and sensitivity of bacteria for 24hrs. Results: The study showed that highest number of patients having Klebsiella pneumonia were from 50-70 years having 20 (40%)patients followed by 30-50 years with 16 (32%), from Above 70 years 12 (24%) and below the age of 30 years having lowest number with two (4%) patients out of all patients. The number of male patients

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33(66%) is more than females 17(34%). In our study, we collected 1133 samples during periods of 9 months and out of which 477 samples were positive. Furthermore, 50 (10.48%) samples were positive with Klebsiella pneumonia out of 477 total positive samples. We collected different types of samples for Klebsiella pneumonia including urine sample 34(68%), sputum 10(20%), blood 4 (8%) and pus swabs 2(4%). The most of the antibiotics like Amikacin, Gentamicin, Imipenem, cefazolin etc were resistant while by manual method there was 100% sensitivity with Teigocycline, Colistin 90%, Fosfomycin 88%, while both nitrofurantoin and netilimycin 6% sensitivity. Conclusions: The majority of the K. pneumoniae isolates were resistant to a variety of antibiotics. The majority of them were also revealed to be biofilm makers in varying capacities. Global efforts should be stepped up to limit the spread of multi-drug resistant germs and remove hospital-born microorganisms, which are causing a substantial increase in mortality.

***Keywords*---**klebsiella pneumonia, resistance, sensitive.

Introduction

Klebsiella pneumoniae is a Gram-negative bacteria and an important opportunistic pathogen that usually causes nosocomial infections in hospitalised or otherwise immunocompromised patients, causing significant morbidity and death.[1] At the moment, K. pneumoniae has great resistance to a wide range of medicines. [2] As a result of this resistance, there is a rising global problem with the selection of appropriate antibiotic therapy for hospital-acquired illnesses.[3] Antibiotic resistance has become a concern for doctors and other infection control agents in their endeavour to cure and prevent illnesses caused by microbes long considered to be destroyed by antimicrobials.

These germs, known as superbugs, are resurfacing in novel forms that are resistant to nearly all therapeutically significant antimicrobials. Unfortunately, the pharmaceutical industry does not produce enough new treatments to keep up with drug-resistant bacterium infections.[4] Antimicrobial resistance is frequently associated with the expansion of transmissible plasmids and the acquisition of resistance genes, which normally occurs by horizontal gene transfer and may also carry virulence determinants.[5] The development of resistance and virulent features is required for pathogen survival, and some data show that such traits may play an important role in the pathogenesis of K. pneumoniae infections.[6,7]

According to Shiri et al.[8], this bacterium is responsible for approximately one-third of all Gram-negative infections, including urinary tract infections, cystitis, pneumonia, surgical site infections, endocarditis, and septicemia. Necrotizing pneumonia, pyogenic liver abscesses, and endogenous endophthalmitis are also caused by it.[9] The dramatic increase in the occurrence of multidrug-resistant (MDR) and exceptionally drug-resistant (XDR) Enterobacteriaceae infections is a big economic challenge since these germs are common natural inhabitants of

human and animal microbiomes. Despite its multiple clinical implications, there is currently a scarcity of data on K. pneumoniae.

In the hospital context, K. pneumoniae is also the most prevalent multidrug-resistant and carbapenem-resistant infection. Polymyxins have been the most often employed antibacterial alternatives against carbapenem-resistant K. pneumoniae throughout the previous few decades. Indeed, polymyxin E (colistin) has been regarded as a "last option" antibacterial in the battle against MDR K. pneumoniae infections, frequently serving as the only antimicrobial capable of achieving acceptable serum levels and minimum inhibitory concentrations (MIC). [12] As a result, new reports of colistin-resistant K. pneumoniae isolates are concerning, given the further limits of antibiotic choices and the high death rate associated with these infections.

Colistin V/S K. Pneumonia

Colistin (Polymyxin E) is a polymyxin-class cyclic polypeptide bactericidal antibacterial with focused Gram-negative action. Colistin's chemical structure is similar to that of other antimicrobial peptides generated by eukaryotic cells, such as defensins, and its unique tri-dimensional structure enables at least three separate antibacterial processes.[9-11] Despite its high bactericidal efficacy, colistin usage is frequently linked with important adverse effects, such as nephrotoxicity and neurotoxicity, which have been recorded in 14–53% and 4–6% of patients, respectively.[13–15]

The precise processes producing these adverse outcomes are unknown, however colistin's hydrophobic characteristics may explain them. Until recently, colistin was thought to be a "last option" antibiotic for treating infections caused by carbapenem-resistant K. pneumoniae. Unfortunately, as the usage of colistin has increased, the occurrence of colistin-resistant K. pneumoniae has been recorded. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) defined in vitro colistin resistance for K. pneumoniae as a minimum inhibitory concentration (MIC) of more than 2 mg/L, and recommended determining colistin MIC by broth microdilution.[16] Furthermore, outbreaks of colistin-resistant K. pneumoniae have been documented in the United States, Canada, South America, and Europe.[17] Recent findings show that resistance to colistin is present in up to 43 percent of carbapenem-resistant K. pneumoniae in Italy, 20.8 percent in Greece, and up to 31 percent in Spain.[18-19]

Tigecycline and K. Pneumonia

Tigecycline is an antibiotic with a broad range that belongs to a novel class known as glycylcyclines. Glycylcyclines are a homologue of minocycline and exhibit action against a wide range of bacterial infections.[20] Tigecycline is the first of a new family of antimicrobials with additional qualities that negate the majority of mechanisms driving tetracycline resistance. In vitro testing has revealed that tigecycline has activity against vancomycin-resistant enterococci, methicillin-resistant Staphylococcus aureus, penicillin-resistant Streptococcus pneumoniae, and many species of multidrug-resistant Gram-negative bacteria, though Pseudomonas aeruginosa resistance and reduced susceptibility among Proteus

species do occur. Tigecycline looks to be a promising new broad-spectrum antibiotic. Tigecycline resistance in K. pneumoniae is becoming more common in European nations, with a non-susceptible frequency ranging from 7.5 to 50 percent. According to reports from North America, South America, and Asia, the non-susceptible prevalence is 10%.[21] In terms of drug-resistant K. pneumoniae bacteremia, carbapenem-resistant K. pneumoniae bacteremia has a death rate of 40–50% [22], while ESBL-producing K. pneumoniae bacteremia has a mortality rate of 20–30%.[23]

Fosfomycin and K. Pneumonia

Fosfomycin is a phosphonic acid-derived antibiotic with bactericidal activity against Gram-positive and Gram-negative bacteria that is time-dependent. It acts by inhibiting the enzyme responsible for the synthesis of peptidoglycan, which is the major component of the bacterial wall. Fosfomycin has the potential to be a drug against MDR bacteria, particularly when combination with other medications. It belongs to the epoxide class of antibiotics, to which no other antibiotic now belongs.[24] Fosfomycin inhibits the formation of N-acetylmuramic acid after entering the cell wall via the alpha-glycerol-phosphate and glucose-6-phosphate transport systems, resulting in bacterial mortality. Fosfomycin has a low molecular weight and a simple structural structure, as well as an outstanding safety and tolerability profile; it does not induce nephrotoxicity or hepatotoxicity. However, because a substantial quantity of salt is supplied with every gramme of fosfomycin, a regular assessment of serum electrolytes is recommended during fosfomycin treatment.[25]

Material and Methods

After ethical approval from the institutional ethical committee this study was done in the department of microbiology from April 2021 to march 2022 in genesis institute of dental science and research centre with collaboration of anil baghi hospital, firozpur, Punjab India. Demographic profile of all the patients like age, gender, history of any diseases was noted. All the sample like urine, sputum, blood, pleural fluid and urethral discharge were collected in the department for isolation and identification of K. pneumoniae. Samples were inoculated on MacConkey agar culture media plates, and then culture media plates were incubated for 24hrs for growth of any bacteria. After 24hrs those were positive sample, further proceed for grams staining. B D Phoenix advanced automated microbiology system was used for identification and sensitivity of bacteria for 24hrs. For manual evaluation of the sensitivity and resistance of K. pneumoniae disc diffusion methods of antibiotics were used. In disc method a limited amount of culture spread on Mueller hinton agar media and standardized antibiotic disc is placed on the plate surface and incubated the culture media plate for overnight. After 24hrs it takes to grow the microbes, if the antibiotic is able to prevent the growth of the microorganism, it does not grow around the bacterial disk, means it's sensitive if the microorganism grows near the antibiotic disc, means organism resistance for this antibiotic. Detection and differentiation of sensitive and resistant conditions from each other was performed based on the diameter of the around the colony as millimetres.

Following manual antibiotic were used in this study

Imipenem, Meropenem, cefepim, Ciprofloxacin, Amikacin, Ceftazidime, Ceftriaxon, Cefotaxime, Ampicilin, Colistine , Fosfomycin and tigecyclin.

Statically analysis

For statically analysis SPSS version 25.0 were used.

Results

The study showed that highest number of patients having Klebsiella pneumonia were from 50-70 years having 20 (40%)patients followed by 30-50 years with 16 (32%), from Above 70 years 12 (24%) and below the age of 30 years having lowest number with two (4%) patients out of all patients. The number of male patients 33(66%) is more than females 17(34%) shown in Table1.

Table 1. Demographic profile of the patients with Klebsiella pneumonia

Age	Number of patients	Percentage
Below 30	2	4
30-50	16	32
50-70	20	40
Above 70	12	24
Gender		
Male	33	66
Female	17	34

In our study, we collected 1133 samples during periods of 9 months and out of which 477 samples were positive. Furthermore, 50 (10.48%) samples were positive with Klebsiella pneumonia out of 477 total positive samples shown in table 2.

Table 2. Prevalence of Klebsiella pneumonia

Prevalence	Number	Percentage
Total sample collected during the study periods	1133	100
Positive sample	477	42.10
Prevalence's of Klebsiella pneumoniae from the total sample	50	4.41
Prevalence's from the positive sample	50	10.48

We collected different types of samples for Klebsiella pneumonia including urine sample 34(68%), sputum 10(20%), blood 4 (8%) and pus swabs 2(4%) shown in Table3.

Table 3: Distribution of samples

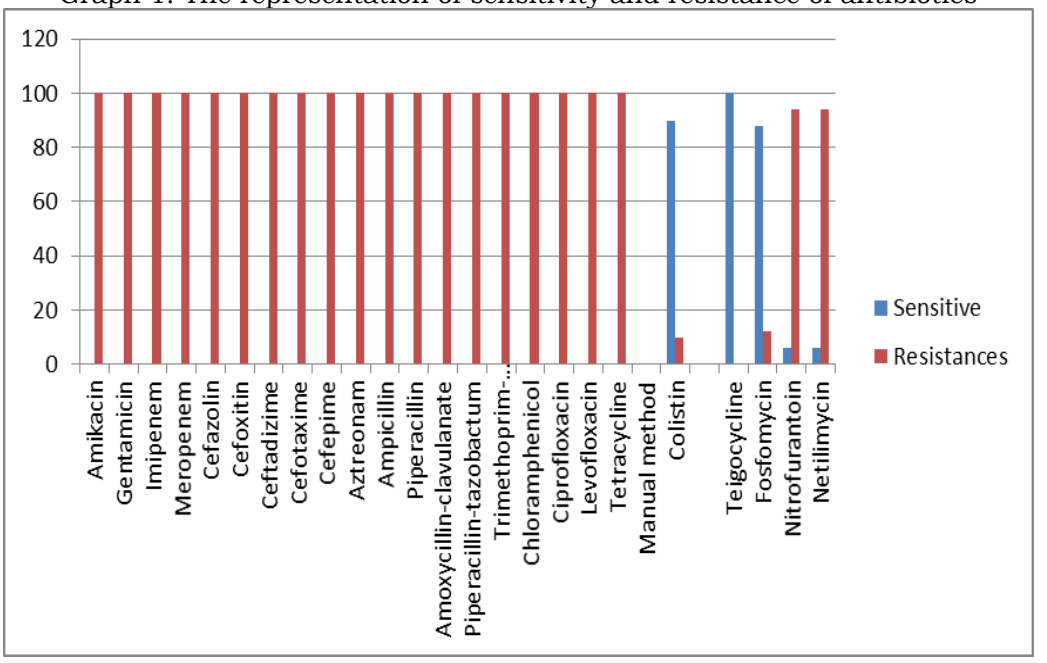
Sample	Number	Percentage
Urine	34	68
Sputum	10	20
Blood	4	8
Pus swabs	2	4
Total	50	100

The most of the antibiotics like Amikacin, Gentamicin, Imipenem, cefazolin etc were resistant while by manual method there was 100% sensitivity with Teigocycline , Colistin 90% , Fosfomycin 88%, while both nitrofurantoin and netilimycin 6% sensitivity shown in Table4.

Table 4. The sensitivity and resistance for antibiotics

Antibiotic	Average MIC	Sensitive	Resistances
Amikacin	>32	-	R (100%)
Gentamicin	<=8	-	R (100%)
Imipenem	<=2	-	R (100%)
Meropenem	8	-	R (100%)
Cefazolin	2	-	R (100%)
Cefoxitin	>16	-	R (100%)
Ceftadizime	8	-	R (100%)
Cefotaxime	8	-	R (100%)
Cefepime	>32	-	R (100%)
Aztreonam	>16	-	R (100%)
Ampicillin	16	-	R (100%)
Piperacillin	>16	-	R (100%)
Amoxycillin-clavulanate	>64	-	R (100%)
Piperacillin-tazobactum	16/8	-	R (100%)
Trimethoprim-sulfamethoxazole	<=4/4	-	R (100%)
Chloramphenicol	>2/38	-	R (100%)
Ciprofloxacin	8	-	R (100%)
Levofloxacin	>2	-	R (100%)
Tetracycline	>4	-	R (100%)
Manual method			
Colistin	4.5	45(90%)	R(10%)
Teigocycline	6.5	50(100%)	-
Fosfomycin	5.0	44(88%)	6(12%)
Nitrofurantoin	3.4	3(6%)	47(94%)
Netilimycin	3.5	3(6%)	47(94%)

Graph 1. The representation of sensitivity and resistance of antibiotics



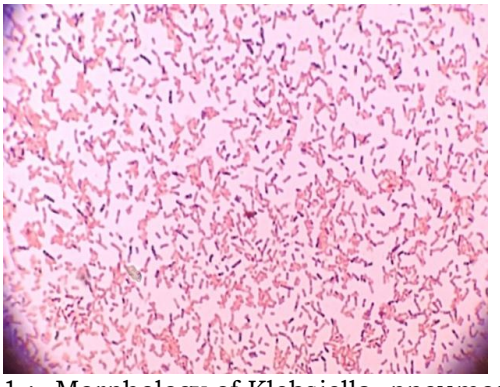


Fig 1 :- Morphology of Klebsiella pneumonia

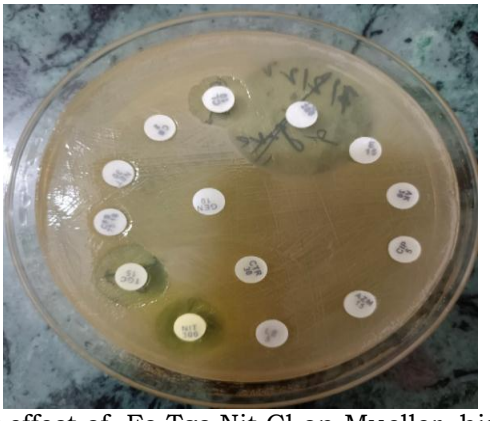


Fig 2. Sensitivity effect of Fo,Tgc,Nit,Cl on Mueller hinton agar media

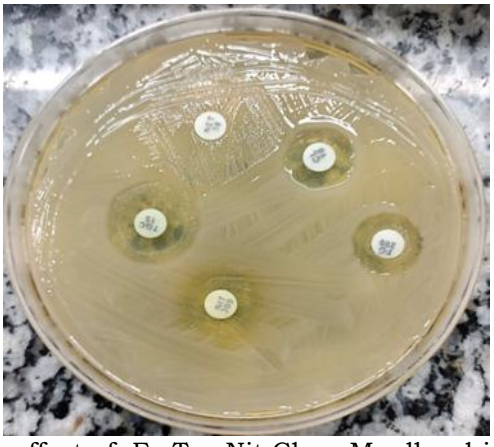


Fig 3. Sensitivity effect of Fo,Tgc,Nit,Cl on Mueller hinton agar media

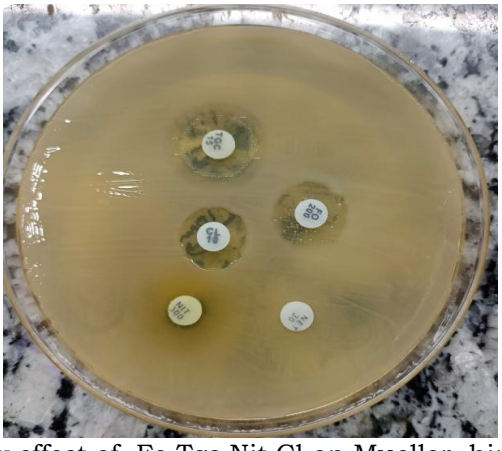


Fig 4. Sensitivity effect of Fo,Tgc,Nit,Cl on Mueller hinton agar media

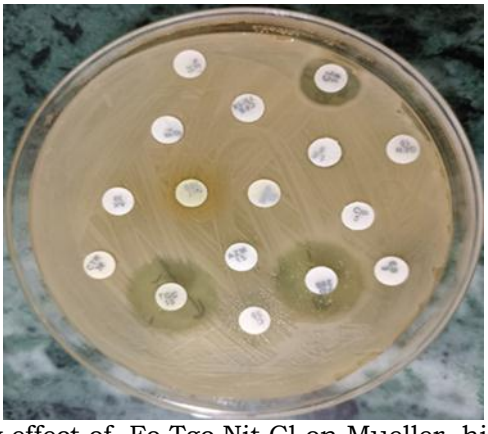


Fig 5. Sensitivity effect of Fo,Tgc,Nit,Cl on Mueller hinton agar media

Discussion

A research looked at the antibiotic susceptibility profile of 1805 Klebsiella pneumoniae isolates from the Hospital Universitário de Santa Maria in Brazil. Resistance to colistin (239.3 percent), ciprofloxacin (64%), and amikacin (21.4 percent) was discovered to be elevated.[26] The sensitivity rate against amikacin was the greatest in the current research. In addition, the sensitivity rate to ciprofloxacin (67.6 percent) was higher than in the previous trial. Another research found that 72.0 percent of the 168 K. pneumonia isolates tested positive for ceftazidime, nearly 69 percent for cefotaxime, and 67.2 percent for amikacin. In our investigation, the sensitivity to amikacin was 100 percent, and there was no resistance to it.

In a study of Klebsiella pneumoniae isolates from hospitals in Sari, Mazandaran, the highest resistance to cefotaxime (100%) and ceftazidim (100%) was found among all isolates, whereas the highest sensitivity to gentamycin was found (63 percent).[27] However, in the current investigation, amikacin and clarithromycin were shown to have the highest antibiotic sensitivity and resistance in Klebsiella pneumonia. A study was carried out to look at the incidence of K. pneumoniae isolates and their antibiotic susceptibility pattern. The most K. pneumoniae were resistant to ampicillin (75.6 percent), followed by nitrofurontoin and cefuroxime (both about 74 percent), and chloramphenicol (the least) (13 percent). [28] Amikacin had the highest sensitivity in the current investigation.

The majority of the K. pneumoniae isolates in our investigation were from male patients. This finding was consistent with the findings of Osagie et al.[29], who collected samples from 5 primary health care clinics in Nigeria and found that K. pneumoniae infection was more common in males than females. According to Akter et al.30, men patients were more likely than females to become infected with Klebsiella. The link between sex and the occurrence of K. pneumoniae was shown to be connected with bad lifestyle choices such as smoking and drunkenness.[29] Those investigations, however, found no statistically significant differences between female and male individuals. The majority of K. pneumoniae in this investigation was acquired from individuals ranging in age from 18 to 65 years.

Meanwhile, another recent study found that patients aged 40 to 65 years old had a higher frequency of K. pneumoniae isolates. 31 The disparities in patient age distribution may be connected to the intensity of the immune system response, which is predicted to deteriorate with ageing. Patients under the age of 40 have stronger immune systems, putting additional strain on K. pneumoniae to battle the host's defence. 32 On the contrary, increasing age increases the risk of K. pneumoniae infection due to a rise in the incidence of concomitant disease. K. pneumoniae isolates 33,34 were primarily obtained from respiratory specimens. According to Ashurst and Dawson, K. pneumoniae commonly colonises human mucosal surfaces of the oropharynx and gastrointestinal system.

As a result, K. pneumoniae is regarded as the leading cause of hospital-acquired pneumonia in the United States. This conclusion is comparable to that of Wang et al.35, who discovered that the respiratory system was the primary location of K.

pneumoniae infection in the Republic of China. In comparison, Seifi et al.[36] reported that K. pneumoniae samples were isolated from urine, surgical wounds, sputum, and blood with a percentage of 61.7, 18.1, 11.7, and 8.5 percent, respectively, from samples collected from two hospitals in Tehran.

The majority of K. pneumoniae was resistant to several medicines, with ampicillin, cefazolin, and cefuroxime being the least effective, while amikacin, piperacillin-tazobactam, and meropenem having the best profile. This finding is backed by a research done by Madahiah[37], which discovered that K. pneumoniae isolates were 100% resistant to ampicillin but 100% susceptible to amikacin. Resistance to ciprofloxacin and amoxicillinclavulanic acid was 38.75 percent and 36.69 percent, respectively. This conclusion is consistent with the findings of Cepas et al[38], who discovered that 40% of K. pneumoniae strains were resistant to ciprofloxacin and amoxicillin-clavulanic acid. The most important element in antimicrobial resistance is antibiotic exposure.

Antibiotic resistance is being caused by a variety of reasons, including the use of antibiotics in hospitals, communities, and even animal production, agriculture, and the environment. As a result of the accessibility to get antibiotics without a prescription, antibiotics are utilised extensively. In the health-care context, extensive and extended antibiotic usage is most likely the primary underlying cause in the widespread transmission of difficult-to-treat antibiotic-resistant nosocomial infections.[39]

Several investigations have proven that, in the majority of cases, a single antibiotic treatment is insufficient to eradicate biofilm-forming illnesses. As a result, controlling infections with currently available antibiotics and assessing the results have become critical and essential measures for the effective treatment of biofilm-associated illnesses. Because of their high antibiofilm activity inside and outside of living organisms, several studies recommend a combination of antibiotic therapy with macrolides such as erythromycin, clarithromycin, and azithromycin as the main antibiotics for biofilm associated infection due to Gram-negative bacteria. Wu et al.[40] proposed that, in addition to the administration of combined antibiotics, removal of infected foreign bodies and the source of infection, as well as the administration of bacterial quorum sensing inhibitors or biofilm dispersal agents, would result in more effective management of biofilm infections.

The most common reason of incorrect antibiotic prescribing is a lack of knowledge regarding infection and antibiotic use. Adjusting initial antibiotic therapy based on clinical microbiology results is one of the most important steps in antibiotic prescribing. As a result, antibiotic susceptibility testing is critical. Collecting clinical samples prior to antibiotic therapy is also important. Many physicians who administer antibiotics are unsure if their incorrect prescriptions contribute to the development of bacterial resistance. In hospital-based illnesses, adjusting the first antimicrobial treatment based on clinical microbiology results reduces the selection pressure on the bacteria. As a result, it is critical for each hospital to have an antibiotic guideline or stewardship programme in place for all pharmacists and clinicians that is based on the most up-to-date microbiological data. To combat the fast emergence of antibiotic-resistant bacteria, a continual

effort in hospital surveillance, infection control, and clinical audits must be done in combination with this guideline. [41]

Conclusions

The majority of the K. pneumoniae isolates were resistant to a variety of antibiotics. The majority of them were also revealed to be biofilm makers in varying capacities. Global efforts should be stepped up to limit the spread of multi-drug resistant germs and remove hospital-born microorganisms, which are causing a substantial increase in mortality.

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