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Review Article

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Global Prevalence and Genetic Landscape of ESBL-Producing Pathogens: A Comprehensive Analysis Across Continents

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Abstract

Multiple drug resistance to antibacterials, antifungals, antivirals, antiprotozoals, and anti-tumor drugs has exploded in the last decade or two, threatening health and medical care solutions in the current scenario. Among the most concerning manifestations of this crisis are the ESKAPE pathogens, a group of six highly virulent and antibiotic-resistant (Gram-positive and Gram-negative) bacterial pathogens, including Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, And Enterobacterspp. These pathogens cause life-threatening nosocomial or community-acquired infections affecting almost all the major systems of the human body including respiratory, urinary systems as well as skin. The economic burden of antimicrobial resistance is expected to rise by 10-fold to about 10 million by 2050. The capacity of multidrug resistant (MDR) germs to evade antibacterial treatments has impeded global efforts to control them. The study assessed 7 keyword combinations on Pubmed. The research articles in each keyword combination were sorted by continent and chronology. The goal of this review summarizes the prevalence of ESKAPE pathogens in different continents of the world with specific emphasis on ESBL producing resistant pathogens. As observed from the studies in the review, E. coli and Klebsiella specifically pneumoniae accounts for the commonest pathogens in the list with blaCTX, bla SHV and blaTEM as the key players. Therefore, prevalence and the spread of these pathogens along with the associated genes have been compiled across the different continents in the current study.

Keywords: Antibiotic Resistance, ESKAPE, ESBL, Global Effects, MDR.

Introduction

The rise of antibiotic resistance in various pathogens, along with its impact on human health and the economy, has brought attention to six particularly dangerous pathogens, known collectively as ESKAPE. These include Enterococcus faecium & faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp. This combination of Gram-positive cocci and Gram-negative bacilli has been categorized as top priority pathogens by WHO owing to their virulence. While understanding the mechanism of resistance for these pathogens, it has been observed that the production of a specific category of enzymes, commonly referred to as ESBLs (Extended-Spectrum Beta-Lactamases), in 'KAPE'— which includes Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp., the Gram-negative bacilli—is one crucial mechanism. Other mechanisms, such as carbapenemase production, efflux pumps, and porin loss, also play significant roles in conferring resistance. Extended-spectrum β-lactamases (ESBLs) are a well-known group of complex and rapidly evolving enzymes that challenge the major therapeutic compounds and obstruct the treatment of both, nosocomial and community-based infections. It is very clear that molecular profiling of the genes associated with the production of ESBLs in these pathogens is of utmost importance as ESBL-producing bacteria are capable of breaking down a wide range of beta-lactam antibiotics, making infections caused by these pathogens particularly difficult to treat. By identifying and characterizing the specific genes responsible for ESBL production, critical insights into the mechanisms of resistance can be gained that can help in tracking the spread of these genes among bacterial populations and develop targeted strategies to counteract them. Owing to this, multiple studies have been carried out across the world in this regard.

Table 1: The table represents the combination of keywords used for search on PubMed.

S.No.	Title	No. of Articles (PubMed) (Research Articles + Review articles)
1	ESBL and ESKAPE	27 + 3 = 30
2	ESBL and Molecular profiling	343 +5 = 348
3	ESBL + Klebsiella pneumoniae + Molecular profiling	95 + 2 = 97
4	ESBL + Pseudomonas aeruginosa + Molecular profiling	26 +1 = 27
5	ESBL + Acinetobacter baumannii + Molecular profiling	6 + 1 = 7
6	ESBL + Enterobacter + Molecular profiling	27 + 0 = 27
7	ESBL+ ESKAPE + Molecular profiling	0

This systematic review is thus focused on the associated genes, their prevalence and the similarities and differences across multiple continents. A set of 7 keyword combinations were analyzed for this review in March 2022 which are shown in Table 1. The keyword combination searches in Pubmed have suggested that compilation of genes across all the ESKAPE pathogens have not yet been compiled in one document. Instead, the majority of the studies were focused on individual KAPE pathogens with maximum in K. pneumoniae and P. aeruginosa isolates. The reviews were removed from the list and the studies in each keyword combination were segregated on the basis of continents Figure 1 as well as chronology Figure 2. Needless to say, with the drastic rise in the resistant isolates in recent years, the analyses have also been carried out at an extensive rate.

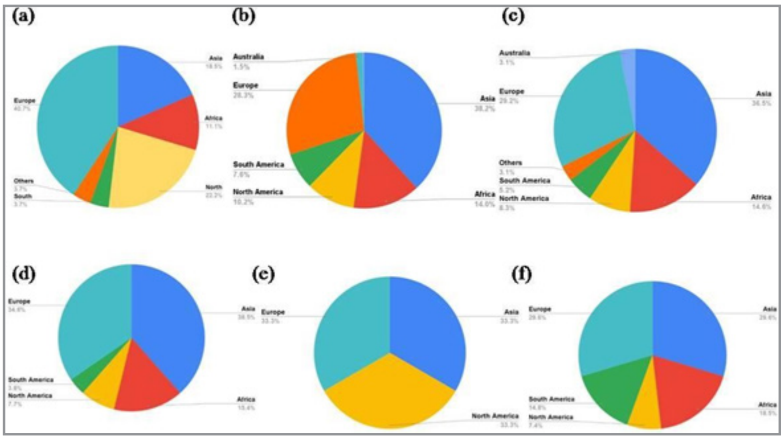

Figure 1: The figure represents the segregation of research articles on the basis of continents wherein the study was carried out for the keywords selected - ESBL and ESKAPE (a), ESBL and Molecular profiling (b), ESBL +Klebsiella pneumoniae + Molecular profiling (c), ESBL + Pseudomonas aeruginosa +Molecular profiling (d), ESBL + Acinetobacter baumannii +Molecular profiling (e) and ESBL + Enterobacter +Molecular profiling (f).

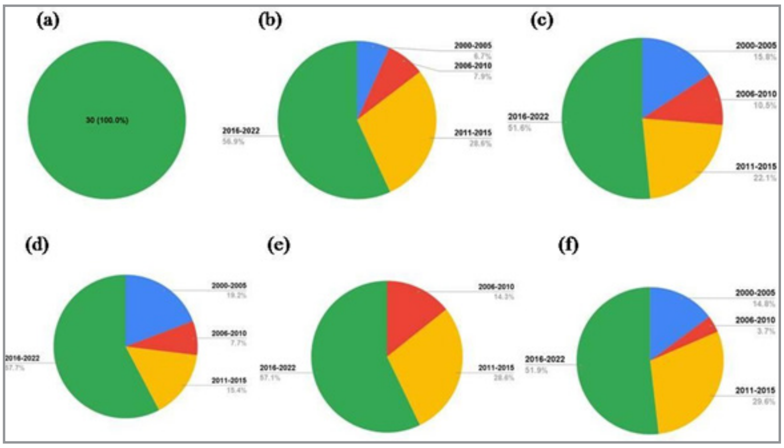

Figure 2: The figure is a longitudinal segregation of the research articles segregated for every 5 years for the selected keywords - ESBL and ESKAPE (a), ESBL and Molecular profiling (b), ESBL +Klebsiella pneumoniae + Molecular profiling (c), ESBL + Pseudomonas aeruginosa +Molecular profiling (d), ESBL + Acinetobacter baumannii +Molecular profiling (e) and ESBL + Enterobacter +Molecular profiling (f).

To further evaluate the status of MDR and ESKAPE in the Indian subcontinent, the studies carried out in Asia were further segregated as Indian vs Non-Indian (Figure 3). It is very clear that the analysis of MDR and ESKAPE pathogens in the Indian subcontinent is very much undermined and thus needs immediate attention. Thus, the review describes the prevalence of pathogenic isolates and their associated genes in different continents.

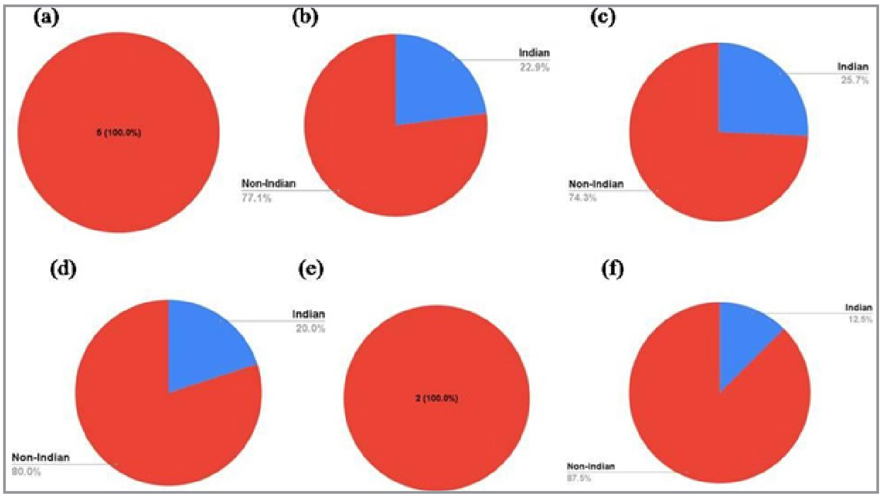

Figure 3: The figure represents the segregation of research studies of Asia in Indian vs non- Indian for the selected keywords - ESBL and ESKAPE (a), ESBL and Molecular profiling (b), ESBL +Klebsiella pneumoniae + Molecular profiling (c), ESBL + Pseudomonas aeruginosa +Molecular profiling (d), ESBL + Acinetobacter baumannii + Molecular profiling (e) and ESBL + Enterobacter +Molecular profiling (f).

Australia

Multiple Gram-negative bacteria are known to produce ESBLs (extended-spectrum-beta lactamase) as a major source of drug resistance such as E. coli, K. pneumoniae, A. baumanii, etc. However, the majority of the research conducted in the Australian subcontinent has been focused on E. coli and species of Klebsiella, of which pneumoniae being the most prevalent one. An association of E. coli infections rather than Klebsiella pneumoniae has been observed with the countries having high prevalence of the infections [1]. Almost all the infections have been associated with nosocomial infections particularly with the neonates [2]. The nosocomial infections caused by these pathogenic species have been associated with multiple routes such as Klebsiella pneumoniae infections via neutropenia and central line infections while E. coli infections with prostate biopsies. The occurrence of these pathogens and the associated genes have been analyzed via multiple methods such as membrane filtration, automated VITEK systems, pulse field gel electrophoresis (PFGE), matrix-assisted laser desorption ionization (MALDI), real time PCR, etc. Discrepancies have been observed in terms of the identification of the Klebsiella species causing these infectious outbreaks in neonates as standard laboratory approaches suggest oxytoca to be the prominent one while whole genome sequencing has claimed michiganensis to be the one [3].

Alternate treatment strategies need to be evaluated for these Klebsiella species isolated from neonates as well as their mothers, owing to them resistance to even the 3rd stage cephalosporins, Ceftriaxone [4]. The spread of this antibiotic resistance could be attributed to a number of parameters including surgical equipment, catheters, etc. Studies have also shown that materials as simple as detergent bottles could be the source of spread of such infections and resistance wherein the transmission route was detergent bottles via milk bottles for neonates and this spread has stopped once the detergent bottles were removed [5].

Detailed analysis of the genes involved in the emerging resistance suggested the key players to be blaCTX-Ms, blaOXAs, blaTEMs and blaSHV. Analysis of E. coli isolates suggested the expression of CTX-M genes in more than 90% of which about 40% was contributed by ST131. On the other hand, analysis of ESBL KP isolates suggested the role of three major clusters including ST20, ST48 and ST1087 which majorly included aac-(6')-Ib-cr and qnrB fluoroquinolone resistance genes. It is imperative to mention here that the associated genes and promoters are both chromosome and plasmid borne. Multiple studies have shown a coordinated interlinking between the promoters present on the chromosome and the plasmid borne genes for the regulation of drug resistance [6].

Africa

In the years August 2011 and May 2014, there was a notable increase in ESBL-producing plasmids among African isolates, rising from 11% to 77% [7, 8]. Isolates of E. coli and Klebsiella pneumoniae that produce ESBL are prevalent due to the presence of high-molecular- weight R plasmids capable of conjugation and transformation. After pneumoniae isolates, oxytoca ranks as the most prevalent species within the Klebsiella genus. E. coli isolates from Water Treatment Plants exhibit resistance to the majority of second- and third- generation antibiotics, particularly cephalosporins, underscoring a significant issue [9]. However, several studies indicate that Pseudomonas aeruginosa is also significant. Non-clinical isolates of Pseudomonas that produce ESBL and MBL demonstrate widespread MBL genes and a notable prevalence of ESBL genes [10]. BlaCTX-M emerged as the primary contributor in ESBL isolates, whereas oprD tested positive for MBL [11]. A study conducted in Nigeria revealed a greater quantity and diversity of enteric bacteria in feces than previously anticipated, indicating clonal proliferation within the Enterobacteriaceae family, known to cause vomiting and diarrhea [12].

In addition to the clinical samples, ESBL containing E. coli have also been found in the food borne isolates in Tunisia responsible for co-morbidities or via poultry foods wherein the poultry is being treated with antibiotics [13]. The analysis of hospital wastewater or feces from surgical units has suggested the role of Enterobacteriaceae family in spreading ESBL resistance. Precisely, hospital wastewater analysis showed the presence of isolates resistant to more than 35% of the antibiotics tested and were thus categorized as MDR [14]. Isolates belonging to 6 different genera of the family were obtained all of which suggested blaCTX-M gene to be the major determinant of resistance [15]. Studies have shown that the beta-lactamases were the most prominent while resistance to carbapenems was very low with multiple genes associated with both the categories [16]. The plasmids consisting of these genes are the major source of transmission across the globe. Certain lifestyle factors such as home interaction with pets, animal food and indoor raised birds further add on to the clonal variety of the blaTEM ESBL types and thus serve as vehicles for undetected clonal expansion of antimicrobial-resistant bacteria [17]. The presence and role of blaTEM gene in emerging resistance has been controversial. A study has shown the presence of the gene at a comparatively low level in the resistant isolates of Enterobactriaceae family including Enterobacter cloacae, K.oxytoca and E. coli while the other suggests it to be a crucial gene for almost all the ESKAPE pathogens [18]. Another study has stated spread of blaTEM to be a major public health concern causing substantial morbidity via Pseudomonas aeruginosa and E. coli isolates [19]. Infact, Pseudomonas species obtained from both clinical and non-clinical sources as well as environmental sources have shown to be resistant to almost all the frontline antimicrobial drugs [20]. Other members of the Enterobacteriacea family such as E. aerogenes and cloacae have also been shown to cause outbreaks of nosocomial infections [21]. Citrobacter and Salmonella spp are other members of these resistant infectious pathogens list. In addition to the ESBL expressing genes, multiple non-ESBL genes have also been identified which are not discussed in this review.

North America

Studies so far have clearly suggested Pseudomonas to be a clinically critical agent owing to its high propensity of MDR as observed with children's patients in Panama with blaTEM to be the key responsible gene [22]. The genes responsible for efflux of the drugs from the cells such as MexAB-OprM have also shown to be ubiquitously present in these isolates [23]. Needless to state Klebsiella and E. coli still remains the most prevalent pathogens showing drug resistance along with its drastic clonal spread in almost all regions of the world and thus remains the most studied ones. ESBL expressing Klebsiella pneumoniae isolates were discovered for the first time in 2001 in New York City with differential sensitivity patterns towards individual and combinational fluoroquinolones, tetracyclines and beta lactamases [24]. It is also presumed that owing to epidemiological interventions, nosocomial transmission had been contained since no such isolates were obtained but that does not seem to be the case during the long run [25]. In fact, to further analyze the severity and the effects caused by ESBL producing Klebsiella isolates, the two categories of isolates (ones producing ESBL vs the other sensitive KP isolates) were analyzed to determine the frequency, risk factors, outcome and molecular epidemiology [26]. It was observed that though there were differences in the two groups in these terms, the difference was not statistically significant [27]. It was also clear that understanding the population genetic structure of strains such as CG258 was essential to determine appropriate targets for treatment options. Analysis of the same revealed certain strains to be virulent around the world and was thus categorized as pandemic [28].

Unlike the other continents investigated previously, investigations involving ESBL genes have been conducted on Acinetobacter spp. within surgical and medical intensive care units. These isolates have demonstrated resistance to multiple antibiotics, including 3rd generation, yet they surprisingly exhibit sensitivity to 1st generation Polymyxin B [29]. Furthermore, E. cloacae isolates have been identified as resistant pathogens in this geographical region, with the blaSHV gene playing a crucial role, followed by blaTEM [30]. Genetic analysis indicates that bla-OXA-51 is a crucial factor in the development of resistance in Acinetobacter isolates [31].

The pathogenic drug-resistant species particularly ESBL and KPC have not only been found in the clinical or community infections but also in wastewater samples owing to themenvironmental spread as well with the prevalence of blaOXA, blaCTX-M and blaTEM genes [21]. Similarly, results have shown that uncooked foods such as iceberg lettuce could be a source of emerging antibiotic resistance wherein drug resistant Klebsiella pneumoniae isolates were identified consisting of blaTEM and blaSHV genes [32].

South America

Studies of isolates obtained from Brazilian hospitals have clearly shown the role of beta lactamases as the principal resistance mechanism [33]. It has also been suggested that multicenter surveillance efforts require epidemiological information which is obtained by phenotypic and genotypic description of the pathogens via multiple techniques such as PFGE [34]. Pathogenic species identification in the South American continent have suggested E. cloacae and E. aerogenes to be the major species with blaCTX-M, blaTEM, blaSHV and blaPER genes to be the major key players of resistance [35]. Similarly, another study in clinical settings has suggested K. pneumoniae, E. coli, Proteus mirabilis and E. cloacae to be the most prevalent species of Enterobacteriacea family on the basis of enterobacterial repetitive intergenic consensus (ERIC) sequences [36]. The study also stated membrane impermeability owing to selective pressure by the hospital environment leading to plasmid resistance as the major cause of antibiotic resistance. Similar results have been observed in another study with isolates from Brazilian hospitals suggesting elevated levels of MICs towards the assessed drugs [37]. The presence of blaCTX-M gene, specifically CTX-M-2 has spread drastically throughout the world after its discovery but the results of the study in this region have shown controversial results. It has been observed that blaCTX-M-1, blaSHV and blaTEM have been present in the resistant isolates across various pathogens with low prevalence of blaCTX-M-2 [38]. Studies have also shown that insertional elements play a key role in the capture, transposition and activation of blaCTX-M genes leading to quick gene dispersion [39].

In addition to the isolates studied from clinical isolates, ones from dairy settings such as fresh feces from cows, rectal swabs, animal hind limbs and bulk tank milk samples were also analyzed. Results have shown the emergence of ESBL producing K. pneumoniae isolates in these samples too with genetic variations in the same with the presence of genes on the mobile genetic elements [40].

Europe

The inter country spread of the French epidemic was evident by the initial spread of A. baumannii infections which were resistant to multiple antibiotics [41]. However, E. cloacae and E. aerogenes were observed to be the commonest causes of the nosocomial infections in the european regions wherein E. aerogenes were re-classified as Klebsiella aerogenes owing to the similarities [42]. As against the usually observed colistin sensitivity in isolates, certain isolates resistant to colistin were observed in these studies for which blaCTX-M-15 and blaTEM are expected to play a key role [43]. The resistant strains obtained during this study in 2011 were found to show similarity to previous strains obtained in 1999 which were responsible for pandemic in France [44]. On the other hand, K. pneumoniae isolates from the neonatal intensive care units have suggested blaSHV-12 gene to play a key role in the emergence of ESBL resistance [45].

One of the most prevalent ESBL producing ESKAPE pathogens, Pseudomonas aeruginosa, was also observed endemic to northern Italy consisting of bla(PER-1) which was observed to be nontransferable owing to its presence on the chromosome [46]. As against the prevalence of OprD gene in the African isolates, its expression was deficient in the European isolates with an overexpression of OprM efflux pumps [45]. Similar to A. baumannii isolates, genetic heterogeneity along with a crucial role of chromosomally located genes in the emergence of resistance was also observed in these isolates. Additionally, CTX-M producing pathogens were obtained in the Greece water bodies suggesting it to be a potential source of resistant isolates and their spread [46].

Asia

Two strains, BP-1(T) and BP-2, of Enterobacteriacea family have been identified to be cephalosporin resistant obtained from nosocomial infections in Pakistan and have been mapped in the phylogenetic tree [47]. A study in Bangladesh has also suggested the presence of resistant strains of Acinetobacter baumannii in nosocomial infections with multiple genes such as blaAmpC, blaOXAfamily, blaKPC etc. to be involved in the emerging resistance [48]. The study also suggested the prevalence of ESBL producing Klebsiella pneumoniae and E. coli strains with the possibility of rodents to be a source of human and animal transmission [49]. However, certain studies have shown lower susceptibility of K. pneumoniae isolates than E. coli towards 3rd generation drugs such as Tigecycline [50]. On the other hand, the presence of ertapenem resistant E. cloacae isolates in healthcare settings in Taiwan have shown the emergence of resistance to be caused by multiple mechanisms such as beta lactamase overproduction, decreased OMP (outer membrane protein) expression and an active efflux pump.

In contrast, studies in Iran and India have found Pseudomonas aeruginosa to be the main carbapenem-resistant beta-lactamase producer, containing blaSHV, blaTEM, blaNDM, and blaVIM genes. Klebsiella isolates from young Bangladeshi children's guts include blaSHV genes. Like prior research, Central China isolates show blaSHV and blaTEM gene predominance. These results contrast those from other continents where blaCTX-M is prominent. In E. coli and K. pneumoniae isolates from Indian tertiary care institutions, blaCTX-M-1 and blaCTX-M-15 genes are likewise correlated. In addition to the most common blaCTX-M-2, Klebsiella isolates from China include blaCTX-M-55, which is projected to be epidemic. However, another Chinese research of intra-abdominal isolates found blaCTX-M and blaSHV-2a genes in ESBL-producing E. coli and K. pneumoniae strains. P. aeruginosa isolates from Malaysian tertiary care institutions harboring gene variations, such as blaGES-13 and blaGES-20, showed similar findings (41). Enterobacteriaceae isolates from Bangladesh have also been shown to have blaCMY-9, a new gene.

In addition to the presence of these isolates in nosocomial infections, these have also been isolated from raw food samples which include K. pneumoniae, E. cloacae and E. coli. Surprisingly, an analysis of ESBL producing K. pneumoniae isolates in hospital settings and wastewater suggested the highest prevalence of these pathogens in wastewater among various sources. Similar to observations in other continents, the most prevalent genes were blaCTX -M, blaSHV and blaTEM while other ESBL producing genes such as blaKPC, blaIMP, blaVIM and blaGIM were not detected in any of the K. pneumoniae isolates analyzed. Similar analysis was also observed when isolates from nosocomial infections isolate particularly HIV patients in South India stating blaCTX-M, blaSHV and blaTEM as prevalent genes while blaOXA genes to be at low levels. Indian studies have also supported a high prevalence of P. aeruginosa isolates in poultry farms as well suggesting it to be a transmission source owing to use of antibiotics in the farms. Similarly, studies from Kerala, India have shown the prevalence of MDR K. pneumoniae isolates resistant to cephalosporins, fluoroquinolones, tetracycline and trimethoprim-sulfamethoxazole drugs in samples from shrimp aquaculture farms. As expected, the genes associated with resistance were located on plasmids, integrons or transposons and were transferred via conjugation. Analyses of streptomycin resistance genes in K. pneumoniae isolates have suggested the transmission of integrons via aadA1 gene cassette. Also, studies have shown the transmission of bla(VEB-1) genes associated with blaOXA10 like genes in Bangkok via class I integrons in Enterobacteriaceae family which are expected to be responsible for resistance to rifampin. Infact a detailed analysis of blaSHV family of genes in Klebsiella pneumoniae isolates in Taiwan have suggested a stepwise evolution of these genes owing to mutations.

Conclusion

As stated earlier, the study involves prevalence of ESBL producing pathogens in different continents. Additionally, the study also highlights the prevalence of genes across the different continents which would be valuable information for analyzing the epidemiology of these pathogens and would thus be crucial in identifying the appropriate diagnostic and treatment strategies. Analysis of the known information for the Australian subcontinent suggested the prevalence of E. coli and Klebsiella infections particularly pneumoniae. Similarly, the gene analyses suggested blaCTX-Ms, blaOXAs, blaTEMs and blaSHV to be the key players. The prevalence of these same pathogens was also observed in the Africa region as well but in terms of gene prevalence responsible for resistance involve blaCTX-M, oprD and blaTEM. Also, infections via Pseudomonas, E. aerogenes and cloacae and Citrobacter and Salmonella spp are also on the rise in the African region. Though the two pathogens (Klebsiella and E. coli) remain prevalent in North America as well, Pseudomonas is a critical pathogen in the list involving blaTEM and MexAB-OprM genes. Additionally, infections by Acinetobacter are also on the rise in this region with blaSHV, blaTEM and blaOXA genes. Similar to North America, Pseudomonas infections along with the two common pathogens are the key pathogen responsible for infections. In addition to the common genes including blaCTX-M, blaTEM, blaSHV, blaPER genes were also observed in this region. On the contrary, the most prevalent pathogen in Europe was observed to be Acinetobacter, E. cloacae and E. aerogenes. However, the prevalent genes remain to be blaCTM and blaTEM along with blaPER, OprD and OprM. An extensive analysis for the same in Asia suggested the prevalence of almost all the pathogens belonging to Enterobacteriacea family including Acinetobacter baumannii, Klebsiella pneumonia,E. coli and Pseudomonas aeruginosa. Similarly, when the genes were evaluated for their prevalence, a plethora of genes and their variants including blaAmpC, blaOXA family, blaKPC, blaSHV, blaTEM,blaNDM and blaVIM, blaGES-13 and blaGES-20 were observed which includes certain novel genes as well. It is imperative to note here that the pathogens and the genes shared in this review is not an exhaustive list and there are many other pathogens present along with multiple genes contributing to AMR. The authors in this review have made an attempt to compile the prevalent pathogens along with the prevalent genes in the region and we believe that such an analysis at periodic intervals would be helpful in determining the emergence and spread of resistance genes across different regions in the world.

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Competing Interest

The authors declare that they have no competing interests related to this work. There are no financial, personal, or professional conflicts that could have influenced the content or conclusions of this review article.

Authors' Contribution

TP conceptualized the study framework, gathered relevant literature, and drafted the manuscript; HJ gathered relevant literature, and drafted the manuscript; AK designed, conceptualized the study framework, wrote the manuscript and reviewed content.

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