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CORRELATION BETWEEN VIRULENCE AND ANTIMICROBIAL RESISTANCE TO ANTIMICROBIAL DRUGS IN *KLEBSIELLA PNEUMONIAE*

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ABSTRACT

Klebsiella pneumoniae is accountable for a widespread range of infections such as pneumonia, urinary tract infections, liver abscesses and bacteremia. In addition to susceptible clinical isolates involved in multidrug-resistant (MDR), nosocomial infections and hypervirulent (hvKP) strains have evolved separately in distinct clonal groups. These isolates are spread in various geographical regions globally. However, the virulence of *K. pneumoniae* is still unknown but the virulence of hvKP is beginning to revealed. The antimicrobial resistance is creating threatened for the treatment of *K. pneumoniae*. The antimicrobial resistance is usually associated with genetic mobile elements such as plasmids having virulence determinants. A proficient pathogen should be virulent, resistant to antibiotics, and epidemic. However, the interplay between resistance and virulence is poorly understood. Here, we review current knowledge on the topic.

Keywords: *Klebsiella pneumoniae*, Resistance, Virulence, Correlation.

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INTRODUCTION

Pneumonia caused by *Klebsiella pneumoniae* is the contributing mediator of a diversity of diseases, containing soft tissue infections, urinary tract, pneumonia and bacteremia. In developing countries, *K. pneumoniae* has become an opportunistic pathogen accountable for many nosocomial infections **[1]**. Nevertheless. A unique syndrome of community-acquired invasive disease caused pyogenic liver abscesses has been initiated **[2]**. This disease mostly reported in Asia, but few cases have also been seen globally **[3]**. The isolates belong to hypervirulent (hvKP) mostly serotypes K1 and K2 caused these infections. A particular clone like clonal complex 23 (CC23) belonged to Serotype K1 than ST23 and ST57 **[4]**. Many sequence types (STs), are belong to Serotype K2, and few are associated with hypervirulence such as ST380, ST375 and ST86. The isolates associated with ST65, ST57 and ST375 caused invasive infections **[5]**. In competition, isolated associated with *K. pneumoniae* have attained antimicrobial resistance. For instance, the rates of extensively drug-resistant (XDR), pandrug-resistant (PDR), multidrug-resistant (MDR) isolates were 22 %, 61.4 % and 1.8 % respectively reported in Beijing hospital, China from 2010 and 2011. *Klebsiella pneumoniae* has been becoming resistance to many antibiotics and this is a major problem to eradicate *Klebsiella pneumoniae* from different hospitals. *Klebsiella pneumoniae* belonged to ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*) caused infections in Us hospitals **[7]**. Mostly MDR isolated of K. pneumoniae produced extended-spectrum β-lactamases (ESBLs) and carbapenemases (KPC) in combination with aminoglycoside and quinolone resistance, belonged to many clones such as CC258 in contrast with ST11, CC14, ST258, CC15, ST340 and ST512 **[5]**. hypervirulent species and MDR are overlapping species for a long time but few cases have been reported recently **[8]**. The organisms producing ESBL were detected in Europe and then France. The *Klebsiella* belonged to ESBL ranged low in Sweden 3% but 34% high in prevalence intensive care units (ICUs) in Portugal. About 6.1% isolates of K. pneumoniae in North America from ICUs showed resistance to third-generation cephalosporins. In South African hospital, *K. pneumoniae* produced

ESBL were 36.1% but the percentage is about 5% in Australian hospitals but 30-60% of Klebsiella were found in Colombia, Venezuela, Brazil. But the rate of ESBL producing *K. pneumoniae* low in Japan 5% than in Asia 20-50% **[9]**. The positive strains of KPC have spread internationally. In some countries such as Colombia, Israel and Greece cases are endemic but in other countries such as New Zealand, Australia and Canada, they are imported from other countries **[10]**. The *K. pneumoniae* virulence factors are lipopolysaccharides, capsule fimbriae and siderophores (*salmochelin, enterobactin, yersiniabactin and aerobactin*) and efflux.

Some factors, such as fimbriae, capsule, enterobactins, and biofilm formation, are found in almost all isolates and seem to be at the origin of classical pathogenesis. A number of putative virulence factors have been associated with hvKP, while CC258 is almost entirely devoid of virulence genes. The strains of hvKP contained aerobactin and RmpA (a mucoid phenotype). Virulence plasmid encoded by this plasmid **[11]**. Besides, yersiniabactin (iron acquisition systems) encoded by conjugative and interrogative element (ICE) ICEKp1 **[12]** and allantoin metabolism regions associated with strains of hvKP **[13]**. the procurement of clusters of the siderophore of K. pneumoniae has elevated the risk of serious infections in humans **[14]**. *K. pneumoniae* can attained antimicrobial resistance by mutation in DNA or by horizontally gene transfer **[15]**. Nevertheless, acquirement of resistance against antibiotics transfer fitness cost. These reduces the inexpensive characteristic of bacteria in the nonappearance of antibiotics. Mutations and deletion in genes associated on chromosomes involved in antimicrobial resistance, initiates fitness cost. The acquisition in plasmid the cost is lower **[16]**. Plasmids play very important role in acquisition and dissemination of determinants cause resistance and virulence genes *in K. pneumoniae. Klebsiella pneumoniae* showed permeability to plasmids. Mostly strains contained low-copy-number and high-copy-number plasmid in their plasmids **[17]**. The relationship between virulence and resistance is a serious issue due to acquisition of virulence factors, antimicrobial resistance and phylogenetic background. *K. pneumoniae* has genomic diversity with gain and loss of genes due to lateral gene transfer like *Escherichia coli* **[15, 17]**. The main purpose of the review is to discuss virulence and antimicrobial resistance in *K. pneumoniae* strains.

RESISTANCE TO β-LACTAMS

Klebsiella pneumoniae is resistance to β-lactam ring containing broad spectrum antibiotics such as carbapenems, penicillins, monobactams, cephalosporins and β-lactamase inhibitors **[18]**. The main cause of getting resistance in Gram-negative bacteria is the β-lactamase enzymes production with the help of transpeptidases enzymes present in cell wall and excessive release of β-lactam containing molecules. *Klebsiella pneumoniae* adopt the mechanisms of getting resistance due to producing β-lactamase enzyme by efflux pump and changing the cell wall permeability **[19]**.

β-lactamase Expression and Virulence Association

Extended Spectrum β-lactamases

K. pneumoniae produced Extended Spectrum Beta Lactamase (ESBL) which are divided into following derivatives SHV, TEM and CTX. Active sites of ESBLs interchange their amino acids due to mutations in SHV and TEM genes present on plasmid. In 1983, it was reported form Germany that bacteria belong to *Enterobacteriaceae* family may also produce ESBLs **[20]**. Chromosome of *Klebsiella pneumoniae* produced SHV-1 β-lactamase and become resistance to antibiotics carbenicillin and ampicillin. In 1980s, ESBL enzyme that breakdown the oxyimino-cephalosporins was reported **[21]**. Mostly ESBLs are mediated by plasmid and play an importance role in hydrolyzing antibiotics. First, second, and third-generation cephalosporins, penicillins and aztreonam resistance is increasing due to breakdown of antibiotics. Other enzymes β-lactamase inhibitors stop the steady increasing resistance but cannot work against carbapenems and cefoxitin because their plasmids adopt other mechanisms for causing resistance to aminoglycosides, Fluoroquinolones and cotrimoxazole. In 1980s, TEM-2 and classic SHV-1 genes were dominant in producing ESBLs due to genetic variants but CTX-M group is new ESBLs producing family in the beginning of the 1990. Nowadays CTX-M enzymes are leading ESBL type but *K. pneumoniae* contained CTX-M-15 enzyme presently observed **[22]**.

Correlation with Adhesions

Klebsiella pneumoniae contained fimbrial and nonfimbrial adhesins for colonization within mucosal tissues of human beings. These are extracellular appendages contained thousands of protein subunits. Mucosal surfaces are including gastrointestinal tract especially oropharynx where primary infection appear benign **[23]**. Fimbrial adhesins are KPF-28 encoded by plasmid, E. coli common pilus (ECP), mannose sensitive type 1 fimbriae and type 3 fimbriae. While CF29K include in nonfimbial adhesins **[24]**. *Klebsiella pneumoniae* contained several virulence genes like uge, wabG,

ureA, mrkD, kfuBC, rpmA, fimH and there functions are encode enzyme uridine diphosphate galactouronate 4- epimerase, outer core lipopolysaccharide biosynthesis, relevant to urease operone, mucoviscosity associated gene A, adhesions with type 3 fimbriae, allantoin regulon activation, iron uptake system, mucoid phenotype regulation, and type 1 fimbial encoding gene respectively and are involved in pathogenesis **[25]**.

Other virulence factors produced by *K. pneumoniae* are lipopolysaccharides, siderophores, capsular polysaccharides, fimbriae and serum resistance **[26, 27]**. The link between resistance to β-lactams due to production of ESBL and *k. pneumoniae* adhesions to cell surface were investigated from 1990 and 2000 in several studies **[28]**. In 1965, the first β-lactamase enzyme Temoneria (TEM-1) enzyme was discovered and soon (SHV)-1 sulphydryl variable β-lactamase enzyme was described. Which showed resistance to penicillins and contained ESBLs **[29]**. At this time (TEM-1) (TEM-2), (SHV)-1 was more prominent in ESBLs. Since 1984 study in France, ESBLs producing bacteria belong to family Enterobacteriaceae contained enzymes TEM (TEM-3, TEM-5, TEM-8, TEM16, TEM-24) or SHV-1 (SHV-4). TEM derivatives are encoded by genes present on 85-Kb R-plasmid while SHV derivatives are present on 185-Kb plasmid **[30]**. CF29K nonfimbrial proteins present in CC23clone (80%) and able to cause pyogenic liver diseases it might be caused by other virulence factor present on same plasmid **[31]**. Besides TEM and SHV, CTX-M showed greater activity to cefotaxime than ceftazidime. This plasmid is also responsible to other antibiotics such as trimethoprim, fluoroquinolones and aminoglycosides **[32]**. In the era between 1983-1991, 145 epidemic outbreak occur in which 13 outbreaks were caused by *Klebsiella* **[33]**. Centers for Disease Control and Prevention reported 3% epidemic and 8% endemic outbreaks were occurring by *Klebsiella* spp **[34]**. SHV-5 type of *Klebsiella* strains were commonly resistance to ceftazidime reported in Europe while in United States TEM-10 and TEM-12 were identified **[35]**. The study shown that SHV-4 β-lactamase producing strains of K. pneumoniae were linked with restrict type of adhesion. Same study reported that 45% severe infections were caused by such localized adhesion isolates. As a result, pathogenesis could be caused by such isolates **[36]**. The antibiotic resistance of *K. pneumoniae* strains is associated mainly with the production of ESBL. In 2017 the World Health Organization included ESBL-producing *K. pneumoniae* in the list of the most dangerous superbugs along with *Acinetobacter baumani* and *Pseudomonas aeruguinosa* **[37]**.

Correlation with Capsule Production

Both classical and hypervirulent capsule of klebsiella pneumoniae are made up of K antigens (K1 and K2, upto 78) which is capsular polysaccharide and specific for strains **[38]**. The capsule producing genes are present on chromosomal operons *cps*. The *cps* is the cluster of genes including *wzb, wca, cpsG, wzi, wzc, cpsB, wzb and galF* involved in capsule production **[39]**. *wzi* locus is sequenced by K-antigen typing because it contained genes which encode protein that is involved in attachment of capsule with outer membrane. If this protein is not present results in absence of capsule **[40]**. *Wzy* also called *orf4* and its function is to polymerize capsular polysaccharide and *wza, wzc also known as orf5*, and *orf6* worked as surface assembly. *cpsG and cpsB and cps* gene *encode* phosphomannomutase, mannose-1 phosphate guanyltransferase and capsule polymerization respectively **[41]**.

TEM and SHV type ESBL producing strains of *K. pneumoniae* showed more serum resistance than non- ESBL-producing strains **[42]**. Capsule is a virulence factor and enhance ability to cause diseases because it prevents from phagocytosis. Capsule synthesis and serum resistance properties are correlate with each other. Capsular strains are more linked to cause blood infections. On the contradictory, isolates producing CTX-M showed less serum resistance production in *K. pneumoniae* than non-ESBL-producing strains **[43]**. Two genes *p-rmpA1 (*mucoid phenotype) and gene *p-magA2* (having mucoviosity property) present on two large plasmids **[44]** and one is located on chromosome (c-*rmpA*), involved in capsule production. These genes are present in hv-KP and less prevalence of antibiotics than c-KP strains. 12.6% hv-KP isolates produced ESBLs from invasive infections that was reported in china since 2016 **[44]**. HvKP contained p-*rmpA* instead of p-*rmpA2* or c-*rmpA* when sequenced very first time and involved in enhancing genes to produce capsule. It is more hypervirulent **[45]**. A prevalent study conducted in 2016 demonstrated that 12.6% isolates of hvKP isolated from infections that invade into body organs contained bla_{CTX-M} genes and produced ESBLs. If plasmid is conjugated with blaCTX-M-15 gene, it showed more serum resistance and ultimately higher capsule production than unconjugated plasmid or original host. Isolates having CTX-M-producing genes and ST11 non-ESBL-producing strain showed similarity in serum resistance. Researchers suggested that, traT gene is conjugated into plasmid to attain the property of serum resistance. In another

study, isolates having virulence genes that produced ESBL TEM-47 or TEM-68 caused newborn infections **[46]**.

Correlation with Non-virulence

A study was conducted in 2000 at the teaching hospital of Clermont-Ferrand in France showed that CTX-M-15 produced by *K. pneumoniea* was involved in outbreak **[47]**. Most of the infections occur through anendo scope in which *K. pneumoniea* reside in the form of biofilm. Instead of the presence of virulent capsular serotype K2, *K. pneumoniea* contained some virulence genes but none of the patients were infected. This bacterium can survive in the hospital environment because it has the ability to plasmid transformation into another bacterium. Hence proved that, presence of capsule in not related to virulence. Another study demonstrated that *K. pneumoniae* strains specifically K1 serotype collected between 1917 and 1949 also known as Murray collection showed little or no virulence **[48]**.

Another outbreak of *K. pneumoniae* strain producing CTX-M-15 ESBL was arisen from 2005–2007 in Sweden at the Uppsala University Hospital **[49]**. In this outbreak isolate was colonized and infected about 248 patients. The plasmid encoding CTX-M-15 contained no genes related to virulence but completely adapted the characteristics of its host ultimately have no fitness cost. But in comparison, E. coli received *K. pneumoniae* plasmid contained fitness cost. But it was unstable. The researchers concluded that, CTX-M-15 produced by *K. pneumoniae* plasmid ended in a strain with unique genetic framework but have the ability to cause outbreaks. CC23 strains also contained plasmids that encode CTX-M-15 ESBLs **[50]**.

RESISTANCE DUE TO CARBAPENAMASES

Now-a-days, Carbapenems are becoming a last ray of light in the darkest era of antibiotic resistance against multi drug resistance strains of gram negative rods. These drugs of choice were introduced first time in 1980s **[51]**. The enzymes like carbapenamases which are active against few carbapenems and they have ability to dissociate carbapenems and some β-lactams. There are many classes of β-lactamases **[52]**. The antibiotics like carbapenems and penicillins are hydrolyze by all classes of enzymes. But in the case of cephalosporins, enzyme's mode of action varies with class. The divalent cations addiction of enzymes for its proper functioning is responsible for their classification. The carbapenameses are classified into two groups metallo-carbapenemases which are zinc dependent (class B) and non-metallo-carbapenamases which are zinc independent (classes of A, C and D) **[53]**. The attained class B MBLs consist of VIM, IMP groups along with latest evolving NDM groups are recognized in Enterobacteriaceae family **[54]**. The metallo β-lactams which are broad in spectrum with reference to substrate and they have capability to hydrolyze whole β-lactam antibiotics that must contain carbapenems excluding monobactams **[55].** The New Delhi metallo- β-lactamase NDM-1 is the most important clinically recognized carbapenemases which was first time observed in 2008 in *E. coli* and *K. pneumoniae* diagnosed patient who was coming back from India to Sweden **[56].** The carbapenemases which are related to class A according to Ambler classification system have ability to dissociate all types of cephalosporins and aztreonam as well. The type of carbapenamaese which are active against cephalosporins of first generation,ceftriaxone,cefepime and cefotaxime are OXA. But it remains dormant against azytreonam and ceftazidime **[57]**. The high resistance enzyme *Klebsiella pneumoniae* carbapenemases KPC were evolve first time in *Klebsiella pneumoniae* in 1996 **[58]**. Out of eleven KPC strains which are ranging from KPC-2 to KPC-12, KPC-2 is the most notorious strain. **[59]**. Outbreak of KPC-2 strain of *Klebsiella pneumoniae* in New York in 2004 has been reported **[60]**. The difference of one amino acid converted KPC-2 to KPC-3 and this strain was first time reported in 2000 to 2001 during the outbreak of *Klebsiella pneumoniae* in New York **[61]**. Another report of KPC-2 which was documented in 2005 from France revealed that this strain of Klebsiella pneumoniae has been disseminated in the world **[62]**. The hydrolysis of almost all β-lactams especially of cephalothin, ampicillin, cephaloridine, nitrocefin and benzylpenicillin is the task of KPC related cephalosporins. The minute but countable hydrolyze activity of KPC was noticed against ceftazidime and cefoxitin which place them under the umbrella of broad dissociation pattern of antibiotics which must include β-lactam **[63]**. The types of plasmid encoding β-lactamases which are ACT-1, CMY-1, MOX-1, CMY-2, MOX-2, ACC-1, DHA-2, MIR-1, FOX-5, CMY-12, ACT-3, CMY-8, LAT-1, DHA-3, FOX-1, LAT-2 and LAT-2b belongs to class C also. The oxacillinases (OXAs) are the most familier enzymes of β-lactamases which are belong to class D, because its hydrolysis rate of isoxazolylpencillin oxacillin is much greater than benzylpenicillin **[64]**. Its sub group OXA-48 was diagnosed as the harbour in *K. pneumoniae* in 2003 in Turkey **[65]**. There are other supplemented types of carbapenemases. The enzyme like New Delhi metallo- β-lactamases (blaNMD) was diagnosed from bacterial infected patient of New Delhi, India **[66]**. The type

blaOXA48 was recognize in Turkey which was recover from *K. pneumoniae* bacterium **[67]**. The reasons for outbreaks of KPC is still confused. Siu et al, found no association between virulence factors and KPC which is the interpretation of conjugation experiment between K2 KP with bla KPC gene. A study conducted in PIMS hospital Islamabad, Pakistan explored 30% cases of K. pneumoniae was the shelter of bla KPC-2 gene **[68]**. Which is one of the highest prevalence rate of this carbapenemase enzyme. These resistant strains reaches to the extent of XDR and the action of caphalosporins, carbapenems, tobramycin, levofloxacin, piperacillin tazobactam and aztreonam found nill against these strains. The enzymes of KPC are also resist to other related antibiotics. Few studies reveal the co-existance of blaKPC-2 and blaNDM-1 genes on the same clone of K. pneumoniae isolates. The availability of both irrelevant carbapenemases reveals the high resistance of isolates to large range of antibiotics especially carbapenem **[69]**. The prevalence of *k. pneumoniae* containing bla_{NDM-1} gene was 32.5% in Asian countries. Then, further annual analysis revealed that the rate of *K. pneumoniae* producing bla_{NDM-1} is increasing day by day after 2010 **[70]**. Hence, KPC enzymes are also interlinked with other antibiotics resistance. In this current situation the partnership of these resistance strains with their virulence factors seem unapproachable. De Rosa et al. interpreted some interesting facts which will be difficult to follow in order to restrict the expansion of this bacterium. The bacterium has a tendency to fit in GIT especially in the absence of protective bacteria which is a source of broad spectrum antibiotics intake. The writers highlighted some factors that leads severity of this pathogen like patient risk factors, lack of infection control measures, hub of digestive tract and few treatment issues **[71]**. The writers recommended the solutions of pointed problems are less duration of patient in hospital, measures set up by infection control committees, intake of gentamicin with polymyxin in order to decontaminate the GIT and carbapenem secure policy **[72-74]**. A study duration between 2008 to 2012 claimed, there was not any combination of OXA-48 *Klebsiella pneumoniae* with capsular virulence factor **[75, 76]**. In a research, NDM-1 strain found more virulent strain and it also showed the link with K2 capsule which act as intrinsic virulence factor. The virulence level was checked by using murine sepsis model **[77]**. This study demands more researches because it seemed that the belonging of K2 capsule make it more virulence **[78]**. In contradiction of above study, the strain containing bla_{KPC-2} showed less virulence in *Caenorhabditis elegans* model. It displays not any relation of bla_{KPC-2} strain with any virulence factor **[79]**. Furthermore, another finding was obtained by study on *Galleria mellonella* model. The model shows the contrast results from patients. The model shows more mortality with KPC (-) as compared to KPC(+) **[80]**. Gharrah et al, found the association between ESBL production, non ESBL production and virulence factors of *k. pneumoniae*. He found the interlink of ESBL strain with biofilm formation, serum resistance and *iss* gene. The relation between non-ESBL production and hypermucoviscosity was also reported **[81]**. Another study is persuasive to some extent about correlation between virulence and resistance of *Klebsiella pneumoniae.* The presence of *rmpA* gene was recorded in two ESBL producing strains which were isolated from septic blood of neonates and these cases was mortal. The mortality may show the severity of disease because of the combination of both virulence and resistance **[82]**. The partnership between anti-microbial resistance and biofilm formation is synergistic to some extent in the nosocomial infection. Vuotto et al, claimed the presence of fimbrial adhesion genes which are responsible for biofilm formation in medical devices have a parallel relation with antibiotic resistance. This appears how the intrinsic resistance manipulate the severity of disease with the antibiotic resistance in hospital environment as a nosocomial pathogen. Another study exhibits the denial homogeneity between multi drug resistance and biofilm formation in nosocomial infection. Consequently, all the isolates were multi drug resistance but shows less level of biofilm formation. Hence proved, multi drug resistance is a key factor for nosocomial infection as compared to biofilm formation **[83]**. Deviated data for relation of virulence with resistance has been observed in hospital acquired infections. But, different studies illustrate the trend of resistance with virulence is abrupt. The light of some studies brighten the concept of correlation of resistance with virulence to some extent. The severity of disease with the combination of these two factors in some studies give a hint for more upcoming researches.

Correlation of Bacterial Outer Membrane protein with Virulence

The Gram negative bacterial outer membrane have some porins which are responsible for influx of hydrophilic substances like antibiotics, nutrients, ions and efflux of toxic materials from bacterium **[84]**. The outer membrane proteins can act as highly immunogenic candidate because it has ability to bind with C1 q and activate the classical pathway in antibody independent manner which can serve as vaccine development purpose. This vaccine will be helpful to prevent the lethargic challenges from the

similar strain of *k. pneumoniae*. Furthermore, the lipopolysaccharide component of cell membrane is independent of the activity of complement mediated lysis **[85]**. The most emergent ST 258 with its CG 258 clonal group was studied in a study in order to distinguish the novel mutant forms. They found 95 % and 86 % matched sequence with respect to OMP A and OMP 26. The researchers examined the sequence of OMPs like OMP A, OMP 26, OMP 36, OMP 37 and OMP 35. Out of two porins like OMP 35 and OMP 36 of *K. pneumoniae*, OMP 36 deficient strain with additional beta lactamases production leads to high level of carbapenem resistance **[86]**. The MDR strain is the major consequences of both OMP 35 and OMP 36 missing strain with ESBL production. The OMP 36 deficient strain shows high sensitivity to phagocytosis by neutrophils and evaluate Lethal Dose 50 in mouse model as compared to parental strain. This scenario revealed that the OMP 36 lacking strain favors high antimicrobial resistance, increase phagocytic activity by neutrophils and decrease virulence **[87]**. This same result was also observed in *C. elegans* model which is less virulence because of missing of both important outer membrane protein **[88]**. The genotyping of MDR isolates of *Klebsiella pneumoniae* which are recovered from ERIC-PCR resulted with fact of association of virulence and resistance. But, RAPD- PCR analysis of same study revealed that resistance is not have any association with the virulence **[89]**.

The strains which are resistance to choloroamphenicol, cefoxitin and quinolones showed minimum adhesion capacity with Int-407. These strains claimed highest mutation capability **[90]**. The mutation of the porins could ultimately results into permeability of membrane which might be consequent to resistance of cefoxitin. This scenario elaborate that these proteins could be allow the bacteria to bind with surfaces. In order to suffer with antibiotic, the bacterium must be enthusiastic to deprive of certain structural capabilities of these membrane protiens. The shadow of these findings casts the impact of antibiotic resistance collaboration with mutation.

Serum resistance is the virulence property of an organism by which they escape the lytic action of complement system of the normal serum **[91]**. Studies have shown that strains of Klebsiella producing extended-spectrum beta-lactamases are significantly more invasive with more fimbrial adhesions and more resistant to the normal human serum bactericidal effect than nonESBL-producing strains **[92]**.

Correlation between Virulence and Efflux Pumps

The genome house of *K. pneumoniae* construct with efflux system that exports antibiotics and substances such as detergents as well as dyes **[93]**. The resistance to antibiotics ranges from quinolones especially ciprofloxacin, nalidixic acid and some antibiotics like chloramphenicol, cefoxitin, erythromycin and tigecyclin are functionalized by *Klebsiella pneumoniae*, due to decoding of its multidrug efflux system of AcrAB. This system recognized as show resistance against few antimicrobial peptides in lung **[94]**. The *C. elegans* model exhibit the over expression of Acr AB punp has correlation with virulence level **[95]**. Recently, OqxAB, efflux system showed resistance to cefoxitin, ciprofloxacin, chloramphenicol and nalidixic acid **[96]**. This is belonging to locus rarA-oqxABR, in which RarA acts as transcriptional regulator of oqxAB and oqxAB and rarA transcriptionally repress by OqxR **[97]**. The latter repressor is mutated which is the reason for multidrug resistance and the enhanced virulence level of the strain notice in *C. elegans* model **[98]**. The RND efflux pump type, resistance nodulation cell division KexD, from *K. pneumoniae* exhibit their role to multi drug resistance, but its virulence role has not been known **[99]**. Likely, pumps like EefABC, colonize in the digestive tract of murine but not linked with antimicrobial resistance. Furthermore, (MATE) KetM which is multi drug and toxic extrusion was not show correlation with resistance to antibiotics **[100]**.

RESISTANCE DUE TO OTHER ANTIBIOTICS

Resistance against Colistin

The pharmokinatics of the colistin is interaction with lipid Aand disruption of outer membrane. The LPS modification by 4-amino-4-deoxy-L-arabinose in lipid A resulted into resisance against colistin in *K. pneumoniae*. The association of this modification with operon, pbgPE, and it is progressed by PhoPO and PmrAB. The activation due to insertion of PhoO/PhoP, regulator MgrB, proposed as colistin resistance determinant **[101]**. Some strains show the colistin resistance pattern due to three genes such as phoO, ccrAB and mgrB, belongs to regulatory system of two components: ccrAB **[102]**. According to Choi and Ko, colistin resistance due to K. pneumoniae strain, ST23, leads to defects in hypermucoviscous and in vitro fitness, CPS production, and serum resistance **[103]**. In recent, colistin is characterized by MCR-1, which is phosphor ethanolamine transferase, plasmid-encoded. Although this enzyme is occasional in *K. pneumoniae* and its virulence role is unclear **[104]**.

Resistance against Fluoroquinolones

The resistance phenomenon in fluoroquinolones are the outcome of the plasmid mediated mutation because of alteration in *parC* (topoisomerase IV) and *gyrA* (gyrase) genes region "QRDRs" that is quinolones-resistance determinant region, alteration in permeability that leads to porin loss and due to overexpression of efflux that resulted into scanty uptake of quinolones **[105]**. A study in Taiwan documented as, qnr genes are 39% prevalent in strains of *K. pneumoniae* which was isolated from blood of patients **[106]**. The strong association between fitness and resistance of fluoroquinolones has been suggested **[107]**. Hence, it seems that this phenomenon has been associated with elevated efflux pump activity as compared to substitutions of amino acid in resistance regions of quinolones.

WHOLE GENOME SEQUENCING CONTRIBUTION

The whole genome sequencing is a tool that allows bacterial strains characterization in depth and facilitate comparison of nosocomial pathogens and outbreaks. This technique is still in the stream of further exploration but it will give great contribution in future for proper understanding of *K. pneumoniae* strains epidemiology and virulence. Due to this, BIGSdb-Kp, database have been developed as a freely accessible tool. The published data of few studies demonstrated the usage of good sequencing technique, high-throughput, especially to achieve genomes of CC258 strain, which is MDR and CC23 endemic hypervirulent strain. Consequently, CC258 was solely devoid from virulence genes, while strains that are hypervirulent and MDR are found as extremely non-overlapping **[108]**. A study showed that intestinal tract of human is the hub of CC23 isolates. Furthermore, virulence plasmid homologs, which is mapped for aerobactin, two siderophores, salmochelin and RmpA were found in entire hvKP. They have few auxiliary siderophore including colibactin, microcin E492 that are associated with ICE, and yersiniabactin. Such kind of strains that have astounding tendency for genomic plasticity, need to be address by addition/subtraction of segments of genomes in a recombination of events **[109, 110]**.

CONCLUSION

The synthesis of enzymes like carbapenemases, cephalosporins and especially extended-spectrum β-lactamases are the usual phenomena of resistance in *Klebsiella pneumoniae*. The β-lactams are enormously used in therapeutics in humans in recent years. The plasmid that contains genes coding of these enzymes, also contain virulence factors genes. Hence, the appreciation of resistance process is difficult over bacterial fitness cost. The genes coding for ESBL (TEM and SHV types) were studied on virulence plasmid. The bacteria enhance its virulence level by possession of such plasmids. Now a day, epidemiology has converted to ESBLs, especially CTX-M type and dissemination of KPC, *K. pneumoniae* carbapenemases. But, such kind of plasmids that carry these enzymes do not come under the category of fitness but these considered as less virulent. Currently, complexity in clones of multi-drug resistance MDR and hypervirulent hvKP are not overlapping. Hopefully, hvKP stability will not maintain by MDR plasmid, because move towards the super bug emergence. The lots of researches need to be done to completely understand the association between reistance and virulence. Particularly, scanty informations are available on acquisition of bacterial mechanism for antibiotics and fitness cost.

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