ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 9, Issue 4, pp: (56-62), Month: October - December 2021, Available at: www.researchpublish.com

COLISTIN RESISTANCE IN *ENTEROBACTERIACEAE* THROUGH SEWAGE SAMPLES/WATERBODIES

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Abstract: **Colistin is an antibiotic for treating various multidrug-resistant Gram negative bacteria. It is at present the last-line drug for infections caused by Gram negative bacteria. Colistin resistance is a concerning issue because of lack of development of new antibiotics. Bacteria like *Pseudomonas aeruginosa*, *Enterobacteriaceae* members such as *Escherichia coli, Klebsiella* spp. have an acquired resistance towards colistin. Due to the high occurrence of colistin-resistant *Enterobacteriaceae* in waste water and process waters from sources like slaughterhouses, hospitals were considered as the hotspot for the colistin resistances (*mcr*) genes. Clinicians should be aware about the possibility of the development of colistin resistance among many multidrug resistant bacteria via adaptations and mutation mechanisms. Rapid growth of bacterial resistance has made it quite tough for us to depend on the development of new antibiotics for which, logical approaches need to be used for utilizing old antibiotics like colistin, to deal with rising issues of colistin resistance.**

Keywords: **Colistin resistance, *Enterobacteriaceae,* Gram negative bacteria.**

1. INTRODUCTION

Colistin is an antibiotic which is the ultimate drug for treating gram negative multidrug-resistant infections. Originally it is obtained from the spore forming soil bacteria, *Paenibacillus polymyxa*. Molecules which are present in this are the polymyxins, categorized into groups as A, B, C, D and E, from which only the polymyxin E (colistin) and the category polymyxin B are generally used exculsively in humans (Biswas,2012). Colistin is a combination of both E1 and E2 polymyxins. These are the two pentacationic lipopeptides which have bactericidal effects (Biswas, 2012). Colistin is very effective on a broad range of Gram-negative bacteria and is not effective on the Gram-positive bacteria. Colistin is generally used as medication in both humans and animals. In case of humans, colistin is usually used in order to treat infections that have developed the multidrug-resistant activity. It is manufactured so that it could be vein or muscle injected or could be inhaled too, i.e, is colistimethate sodium (Gurjar, 2015). In animals, colistin has broad applications in diverse animal food products like beef, dairy, milk- and meat-producing animals for effectively preventing and treating infections. It is caused by the *Enterobacteriaceae* and various other bacteria which are gram negative. The applications or usage of colistin in animals (used as food) is thought to have created this colistin resistance. With spreading of extremely resistant bacteria like *Enterobacteriaceae* producing carbapenemase, colistin at this phase is a last resort medication we have (Aghapour, et al., 2019). Lack of new gram negative antibiotics, have lead us to a reassessment of the present old antibiotics, so it becomes crucial to do research on this matter. The appearances of colistin resistance in bacteria have been a noteworthy medical and public health issue (El-Sayed, et al., 2020). Frequent side effects of these antibiotics comprise several problems of kidney and neurological issues too (Zafer, et al., 2019). Other grave side effects comprise muscle weakness, anaphylaxis, and diarrhea, bronchioles constriction etc. It is also not clear, whether it is safe or not during the pregnancy period (Loho, et al., 2015). The cytoplamsic membrane breaking normally results in the death of bacterial cells. Colistin was first derived and discovered in the year 1947 and the FDA approval of colistimethate sodium

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
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for the medical usage in America was in the year 1970 and was quite popular. The account of the first encoded plasmid, colistin resistant gene (the *mcr-1*) from *E. coli* was seen in China from the livestock/cattle, trade meat and in the clinical isolates of *Klebsiella pneumoniae* caused serious concerns. Moreover, workers in slaughter houses with exposure to the occupational hazards to various colonized animals infected processed water or the water bodies and the wastewater treatment plants or the WWTPs might be open to the elements to a higher risk (Bierbaum, et al., 2020). Furthermore, because of the insufficiency in the wastewater treatment procedures by in-house and various municipal WWTPs the wastewater from livestock may be a chief route of the distribution of the *mcr-1 gene*-carrying bacteria into our surroundings. The fact of high occurrence of the colistin-resistant genes in the livestock feces is quite prevalent. These bacteria generally get built up in the processed water, waterbodies and wastewater coming out of the slaughterhouses (Bierbaum, et al., 2020). These waterbodies may harbor potential reservoirs which could lead to a wide-spread of colistin resistance to several surroundings ecosystems and water-bodies.

2. MECHANISMS OF COLISTIN RESISTANCE IN ENTEROBACTERIACEAE

Even though the key mechanism of the colistin resistance is not clear, Gram-negative bacteria utilizes many mechanisms in order to defend themselves from the colistin. According to researches, many resistance mechanism of colistin are mainly adaptive in nature which occurs with the in-vitro exposure (Andrade, et al., 2020).The colistin Resistance takes place with the modification of LPS through various ways. The most frequent strategy for colistin resistance is certain alterations of the outer membrane of bacteria via variations of the LPS and decline in the negative charge (Andrade, et al., 2020). Further strategy is the efflux pump over the expression procedure. Another procedure is the capsule overproduction of polysaccharide. Figure-1 shows the mechanism.

2.1. Intrinsic resistance mechanisms

Colistin Resistance takes place quite naturally in *Enterobacterice* through modification of the LPS using substitution of cations (Aghapour, et al., 2019). The system of resistance in *Enterobacteriace* is connected to *arnBCADTEF* and the *eptB* gene expression of the operon. Like this way, the 4-Amino-4-Deoxy-L-Arabinose or the L-Ara4N and phosphoethanolamine or pEtN cationic clusters are used with LPS through this gene and operon correspondingly (Aghapour, et al., 2019). The *Enterobacteriace* LPS possess L-Ara4N and the bacterial genome having *eptC* gene, which is intercede in the LPS modification with PETN. *Enterobacteriaceae* Putative loci contain the *sap* (operon) which encodes a protein transport, *O*-acetyltransferase gene and ATPase, taking part in the amino arabinose biosynthesis. Also, the subsistence of the *rppA*/*rppB* TCS has been revealed to be in a roleplay of activating the Operon *arnBCADTEF*. Likewise, this operon is accountable for colistin resistance intrinsic in *Enterobacteriace*. LPS modification and its chrage causes rise in Colistin affinity and reduce the LPS binding. (Aghapour, et al., 2019)

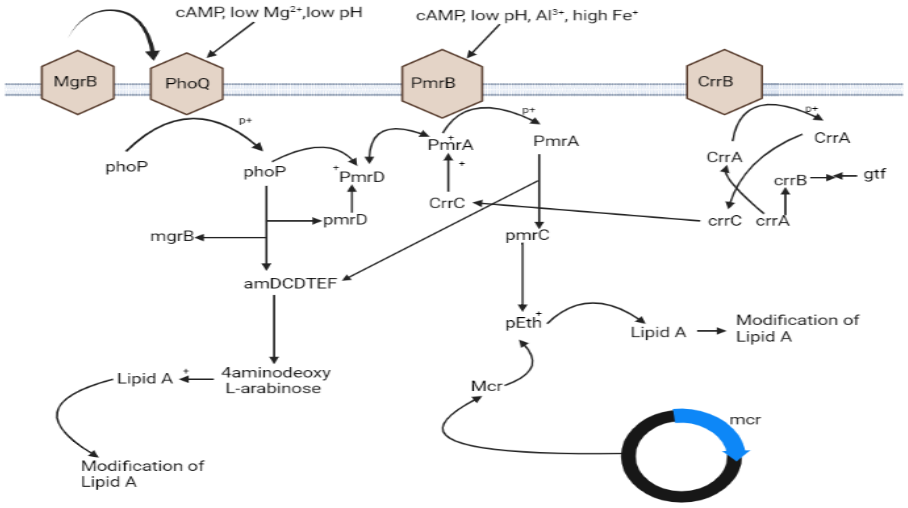

Figure 1: molecular mechanism of colistin resistance in *Enterobacteriace*

2.2. Acquired resistance mechanisms in *Enterobacteriaceaes*

The Acquired mechanism of colistin resistance have been seen in many associates of the members of *Enterobacteriaceae* like, Klebsiella and various other species of bacteria (Olaiton, et al., 2014). Mechanism of resistance is supposed to be linked to untransferable mutations in chromosomes by means of gene transfer horizontally. Only one resistance mechanism/process has been recognized as a mechanism of transfer. Quite a number of operons and genes are involved in the role of LPS modification, which leads to the resistance of colistin (Olaiton, et al., 2014).

2.3 *mgrB gene and PhoPQ and PmrAB regulators of two components systems*

Several regulators and operons play a vital function in modifying the LPS through *PhoPQ* and *PmrAB*. The operon *pmrABC* in the form of regulator protein encodes *PmrA* (*BasR*) as a cytoplasmic membrane bound sensor kinase and the *PmrB* (*BasS*) and *PmrC* as a presumed membrane protein. The adding of the L-Ara4N or L-arabinoseamine to the 4-phosphate or 1-phosphate group causes the colistin resistance (Andrade, et al,. 2020). Usually, L-Ara4N is joined to the 4′-phosphate and it changes it while PETN is linked to the 1-phosphate group (Olaiton, et al., 2014). The operon *pmrHFIJKLM* which is also termed as the *arnBCDADTEF* or the *pbgPE* and PmrE produce L-Ara4N from the acid uridine diphosphate glucuronic acid and then attaches it to the lipid A. The L-Ara4N biosynthesis is dependent on the operon *pmr* (*arn*)(Olaiton, et al., 2014). Furthermore, under various ecological stimulants, like the macrophagic phagosomes, the elevated concentration of iron or Fe^{3+} and aluminium exposure also subject to acidic pH. It causes the activation of *PmrB*. On the contrary, low Mg and Ca concentration causes the *phoQ* activation. Through phosphorylation *PmrB* activates *PmrA* and then *PmrA* in turn, further undertakes activation and regulation of the operons *pmrABC*, *pmrHFJKLM* and the gene *pmrE*. Consequently, these genes and operons causes modification of LPS by adding both L-Ara4N and PETN to the lipid A (Olaiton, et al., 2014). Mutation of the *pmrB*/*pmrA* leads to the *pmrABC* and *pmrFHIJLM* upregulation of operons. *pmrB* and *pmrA mutations* cause colistin resistance, which has been researched in both the *Klebsiella pneumoniace* and *Salmonella entericca*. On the contrary, the *Phop* is encoded by the *phoPQ TCS* in the form of regulator protein and *PhoQ* in the form of a sensor kinase (Olaiton, et al., 2014). Under circumstances of quite low levels of both magnesium or calcium, or cationic antimicrobial peptide, acidic pH, the *PhoPQ* gets activated and it defends the bacteria. Activated *PhoPQ* causes the lipid A modification through two routes.First, activation of *PhoP* by *PhoQ* through its activity of kinase by phosphorylation process. It then activates the pmrFHIJKLM operon transcription, i.e, followed by lipidA modification and *PhoP* in some way that activates *pmrA* via bypassing the connector protein i.e, *PmrD*. Consequently it activates the *pmrHFIJKLM* operon transcription and synthesis of PETN, which moves it to lipid A(Olaiton, et al., 2014) . Many PETN-coding genes, like the *eptA* (*pmrC*), *eptC* (*cptA*) and *eptB* (*pagC*) are quite able to add PETN to various sites of LPS. *phoP/Q* genes mutation has been recognized in *E.coli* and also in *K. pneumoniae* that causes acquired colistin resistance (Olaiton, et al., 2014). The transmembrane protein is encoded by the *mgrB* gene of 47 amino acids which gives negative feedback on the *PhoPQ TCS*. These proteins restrain the *PhoQ* kinase activity, which in return represses the *phoQ* gene expression. However, the *mgrB* mutation causes *phoPQ* operon upregulation and consequent activation of the operon *pmrHFIJKLM*. Lastly, L-Ara4N synthesis causes the lipid A modification and colistin resistance.(F. Andrade, et al., 2020)

2.4. *CrrAB* two-component system

The operon *crrAB* encodes two types of proteins: i.e, the regulatory protein *CrrA* and the sensor kinase protein *CrrB* (Aghapour, et al., 2019). Scientists described that the *CrrB* mutation causes colistin resistance in the *K.pneumoniae*. The *CrrB* mutated protein controls an adajacent *crrAB* gene which encodes a glycosyltransferase protein. It then causes lipid A modification (Aghapour, et al., 2019). In another study, it showed that the *crrB* gene mutation caused the *pmrHFIJKLM* operon activation and activation of the *pmrE* and *pmrC* genes via *pmrAB* operon over expression. Moreover, the L-Ara4N and PETN production and addition to lipid A caused attainment of colistin resistance. It was established that the *CrrC* created a connection between the *pmrAB* and *CrrAB* systems. The *crrB* gene Mutation led to elevated *crrC* transcription (Aghapour, et al., 2019).

2.5. Plasmid-mediated resistance to colistin

Plasmid mediated colistin is quite important issue and also concerning due to easy colistin resistance gene transfer to various strains. The *mcr* genes are accountable for colistin resistance horizontal transfer (Aghapour, et al., 2019). It is worth pointing out that the mcr-1 production causes resistance to lysozymes. Documented findings of the, *mcr1* mediated

colistin resistance genes has been found in many regions, that is, Europe, America, Asia and Africa. Some of the plasmids possesing the *mcr1* gene carry several other genes which are resistant to other antibiotics, like, β-lactams, quinolones, aminoglycosides, tetracyclines, and fosfomycin, etc. The *mcr* gene has also been found in many isolates of *Enterobacteriaceae*, that carry carbapenemase genes as bla_{NDM5}, bla_{NDM1}, bla_{NDM8}, $bla_{OXA49,}$ $bla_{KPC3,}$ and bla_{VIM2}.Recently, scientists dicovered a novel colistin resistance gene of the plasmid mediated ones, i.e, the *mcr2*, in *E. coli* (Aghapour, et al., 2019). Afterward the genes *mcr4* and *mcr3* genes were found. Further, three more varieties of colistin-resistance genes i.e, the *mcr6*, *mcr8* and *mcr7* were discovered in year 2018. Scientists suggested that origins of *mcr-7*, were from the species *Aeromona*, and the structure was quite similar to *mcr3* (Aghapour, et al., 2019). Also it was noted that, *mcr7* showed 78% of the nucleotide identity to the gene *mcr3*. Ultimately, a new genetic factor was found in *K. pneumoniae*. It was recognized as the *mcr8* and its coexistence with the bla carbapenemase gene, was quite concerning. Both *mcr2* and *mcr1* genes origins were from the *Moraxella* spp.(Minarini, 2021)

2.6. Role of regulator RamA

The locus *ramA* has 3 genes i.e, *romA*, *ramA*, and *ramR*. The gene *ramR* has a crucial role as the romA and ramA repressor genes. Some members of the *Enterobacteriaceae* possess a regulator i.e, *ramA*, like the *K. pneumoniae*, *Enterobacter* spp., *Citrobacter* spp and *Salmonella* spp (Aghapour, et al., 2019). In the *Pneumonia* species this regulator regulates the synthesis of lipid A and is linked to several permeability barriers present in it. It has been pointed out that the alterations of *ramA* lead to colistin susceptibility reductions (Aghapour, et al., 2019) .

2.7. Role of capsule in colistin resistance

The function of the capsular polysaccharide or CPS has been confirmed to be defensive against various antimicrobial peptide cations. The capsule layer numbers is proportional to the resistance level. (Andrade, et al.2020) It has been seen that *pneumoniae* species with numerous layers were more colistin resistant than those isolates with less number of layers. Nevertheless, capsular biosynthesis gene upregulation gene caused a decrease in the frequency of colistin interaction with the pneumonia species target-site that was followed by increase in the colistin resistance. As a result, there are few regulators of the capsule formation, like Cpx or the conjugative pilus and Rcs or the regulator of capsule synthesis. Both the Cpx and Rcs also tend to appear in order to contribute for colistin resistance via activation of the KpnEF efflux pump and PhoPQ TCS regulation (Ahmed, et al., 2020).

2.8. Role of efflux pumps

A few reports have showed that these systems of efflux-pump are well involved in resistance against colistin. Many Efflux pumps, like the AcrAB and the Sap proteins, have been found in many species of *Enterobactericeae*. By the pump activation, colistin resistance is increased drastically. (Aghapour, et al., 2019)

3. OVERVIEW OF COLISTIN RESISTANCE ENTEROBACTERIACE IN NATIONAL AND INTERNATIONAL SCENARIO

In a report, the water samples from medical centres and ecological sources that belong to densely populated areas of state West Bengal(India) were studied and *Escherchia coli* was found to be a dominant colistin resistant microbe (Bardhan, et al. ,2020). In second report, colistin resistant microbial strains from the river Funan(located in Chengdu in China) were studied and the microbe was *Enterobacter cloaca* (Tuo, et al.,2018). In third report, in Germany the data were studied from various poultry and slaughterhouses. The bacteria showing colistin resistant was *K.pneumoniae* (Bierbaum, et al. ,2020). In fourth report, in Abboitrs in Germany, the waste water and slaughter house water sources were studied for colistin-resistant genes, the bacteria was K.pneumoniae (Bachmann, et al. ,2021). In the fifth report, throughout the years 2014–2016, from a worldwide study on the *Enterobacteriaceae* included samples from labrotories present in Europe, Asia, Latin America , Middle East including Africa region and laboratories from United States were studied. The dominant colistin resistant bacteria was *Escherchia coli*(Wise, et al. ,2018). In the sixth report, data were studied from several water sources from Nigeria, i.e, Kano, Abuja, Nnewi, Yola , Akwa, Ibadan and Ibom, and the colistin resistant was only found in the *Enterobacteria colaecoce*(Terriera, et al.,2020). In seventh report, from ão Paulo state of Brazil, the data on microbial resistant Enterobacteriace from medical sewage were studied. It was found that the microbe *Staphylococcus aureus* was dominant (Zagui, et al., 2020) in it. In eighth report, village Nguyen Xa (present in province of Thai Binh, country-Vietnam) the waste water from household and clinics were studied and the bacterial culture was,

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International Journal of Life Sciences Research ISSN 2348-3148 (online)
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Escherichia coli. It was also studied that a great fraction of the individuals living in households had members carrying bacteria (with colistin resistant) (Yamamoto, et al., 2018). In ninth report from Africa (North Africa) , the Colistin resistance was seen in isolates of *Escherichia coli* which were studied from various clinical samples (Okere, et al. ,2020). In tenth report, reports from sea food and un-chlorinated water sources of Nepal were studied and the colistin-resistance was found in Escherichia coli (Young, 2019). The tabular format of the case studies is given below (table-1)

Table 1: case studies

Sl. No.	Description	Resistance Microbes	Scientist names
1	Hospital wastewater and ecological sources of west Bengal	*Escherichia coli*	(Bardhan, et al. ,2020)
2	Funan River near Residential areas, China	*Enterobacter cloacae*	(Tuo, et al.,2018)
3	Sewage from poultry and pig slaughter houses, Germany	*K.pneumoniae*	(Bierbaum, et al. ,2020)
4	Wastewater of Abattoirs, Germany	*K.pneumoniae*	(Homeier-Bachmann, et al. ,2021)
5	Exposed wounds of patients infected by infected water , Thailand Malaysia, Spain, italy, Argentina, Germany, Colombia, Brazil, Poland, Hong Kong, Russia Portugal, Taiwan,South Africa and Venezuela,	*Escherichia coli*	(Wise, et al. ,2018)
6	From water areas in Nigeria (i.e, Kano, Abuja, Nnewi, Yola , Akwa, Ibadan and Ibom)	*Enterobacter cloacae*	(Terriera, et al.,2020)
7	Sewage from hospitals, Brazil	*Staphylococcus aureus*	(Zagui, et al. ,2020)
8	Stool specimens of infected individuals working in infected water of agricultural areas, Vietnam	*Escherichia coli*	(Yamamoto, et al. ,2018)
9	Human clinical samples, Africa(mainly from North Africa)	*Escherichia coli*	(Olowo-Okere, et al. ,2020)
10	unchlorinated water and sea-food like shellfish in Nepal	*Escherichia coli*	(Young, 2019)

The graphs below show the frequency of microbial occurrence (the graphs show the number of times these colistin resistant microbes appeared in the study) in percentage are given below in figure-2 and figure-3, with *Escherichia coli* 50% followed by *Enterobacter cloacae (20%), K.pneumoniae (20%) and Staphylococcus aureus* (10%)

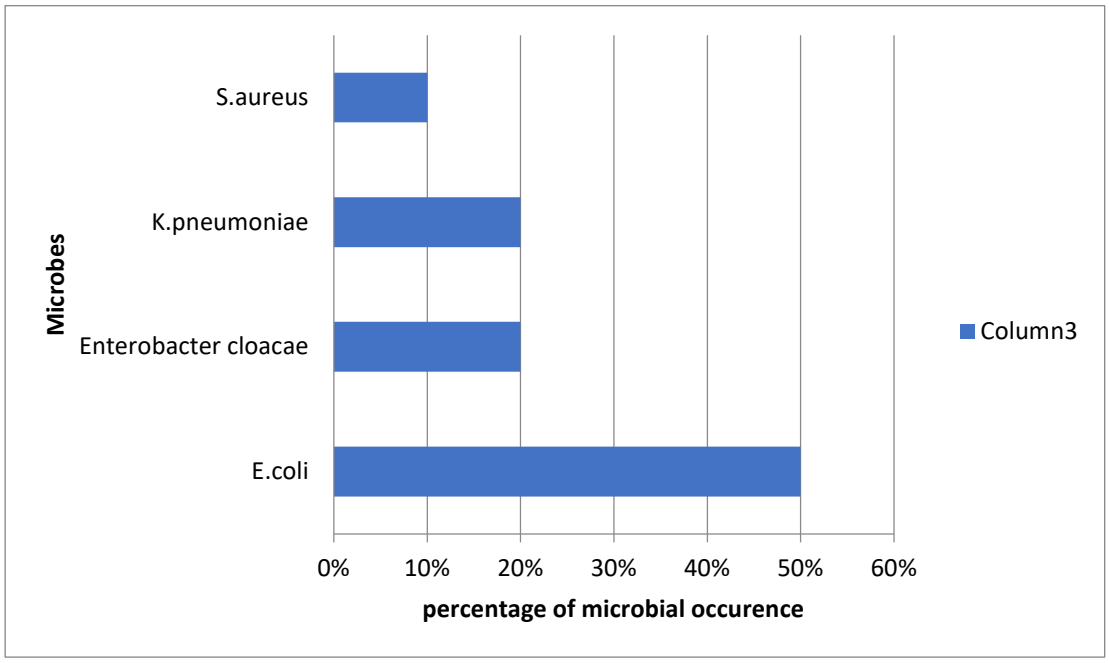

Figure 2: showing frequency of microbial occurance(in percentage) in bar graph

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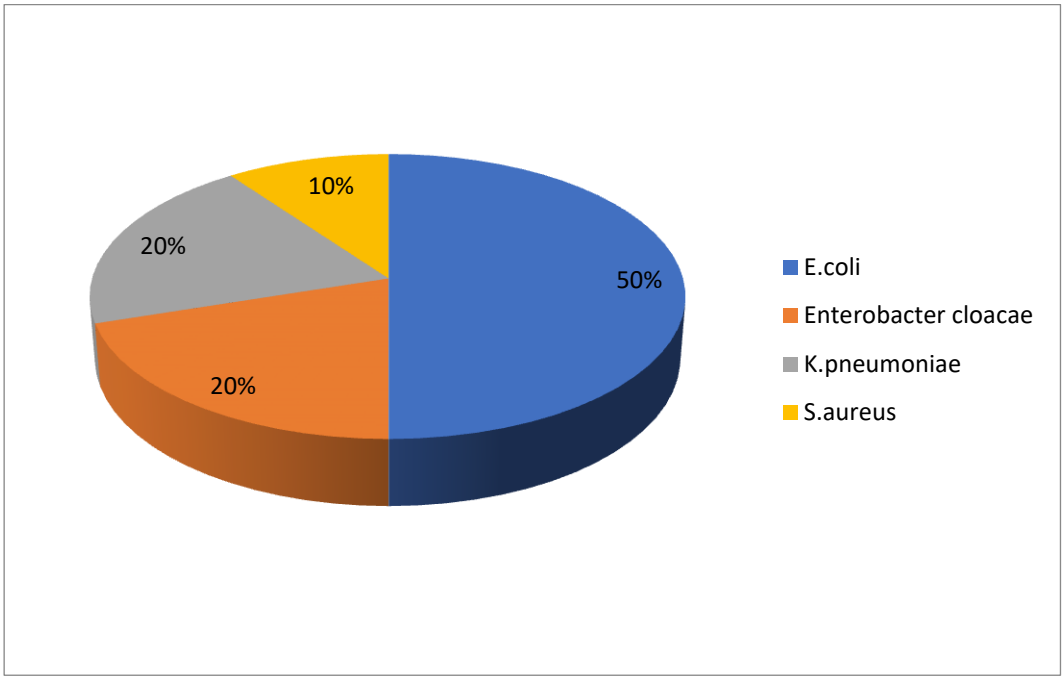

Figure 3: shows the frequency of microbial occurance(in percentage) in pie-chart

4. CONCLUSION

To conclude, from 10 case studies on *Enterobacteriace* with colistin resistant microbes collected, it was seen that the *Escherichia coli* was mostly seen i.e. 50% followed by *Enterobacter cloacae (20%), K.pneumoniae (20%) and Staphylococcus aureus* (10%) . In recent years, the growing use of the colistin in various fields, has caused the surfacing of colistin resistance. Studies have revealed that the cases of colistin- resistance has increased in *Enterobacteriaceae*. Clinicians should be aware about the likelihood of colistin resistance in MDR bacteria and the colistin resistance development via adaptations or mutation mechanism (Shen, et.al.,2020). Rapid emergence of the bacterial resistance has made it tough for us to depend on development of new antibiotics. In this context, we need to take correct approaches for using older antibiotics, like colistin, in order to deal with bacterial resistance.

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