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PATTERN OF CLINICAL PATHOGENS ISOLATED FROM PUS SAMPLES AND THEIR ANTIBIOTIC SUSCEPTIBILITY IN RAWALAKOT AZAD KASHMIR REGION

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Abstract

Despite of advances in limiting the pyogenic infection, diseases have not been solely cured due to mutations, resistance in their genes and many other reasons. The widespread use of antibiotics has led to major problems of producing resistant organisms contributing to morbidity and mortality. This study was aimed to assess pathogenic bacteria and their drug susceptibility patterns from patients with pus and wound discharge. The 100 pus samples from wound infections were obtained at the Sheikh Khalifa Bin Zayed Hospital Rawalakot Azad Kashmir through sterilized cotton swabs. Bacterial isolates were identified by cultural characteristics (colony growth and morphology), Gram and ZeihlNeelsenstaining and Biochemical tests (Catalase, Coagulase, DNAase,Triple sugar iron, Citrate, Indole, Oxidase and Motility). Results revealed that total nine bacterial isolates were identified from the pus samples of patients. *StaphylococcusAureus* was the commonest pathogen seen in majority of the population (49.35%) followed by *Enterococcus faecalis (6.5%), Pseudomonas aeruginosa (16.88%), Acinetobacter baumannii(10.39%),Serratiamarcescens(2.59%), Serratiaodorifera(2.59%), Proteus vulgaris (2.59%), Escherichia coli (3.9%) and Enterobacter cloacae (5.19%).*Then the antimicrobial susceptibility test was carried out by modified disc diffusion method (Kirby-Bauer) recommended by Clinical and Laboratory Standards Institute. The isolates showing most average resistant were of *Enterococcus faecalis(75%)*and most average sensitive isolates were *Proteus vulgaris (70%).* The highest sensitivity rates were observed by most of the bacterial isolates to antibiotics Moxifloxacin, Amikacin, Gentamycin and Meropenem. The overall highest resistant rates were observed by most of the bacterial isolates to antibiotics Amplicillin, Cotrimoxazole and Amoxycillin followed by Ceftazidime, Cefoperazone, Methicillin and Tazobactam. This suggest that formulation of an effective infection control policy is important in preventing wound

infections as well as inappropriate use of antibiotics should be discouraged by the practitioners.

Keywords: Pus, Biochemical Testing, Bacterial Isolates, Antibiotic Sensitivity.

Introduction

Pyogenic infection is characterized by local inflammation, usuallyproduced by one of the pyogenic bacteria and is go along with the dead leukocytes and infectious agent commonly known as pus (Konemanet *al.,* 2005). In the course of pathogenic infection, neutrophils are attracted to site of infection due to cytokines (chemotaxis) released by macrophages. On that spot, the neutrophils tries to kill bacteria by opsonizationand bacteria also secret leukocidin toxin for resistance. As a result of this fighting neutrophils also die off and macrophages eliminate these dead debris in form of pus (Madigan *et al.,* 2006).Several complex factor like carriage of multidrug resistant microorganism, wound become very chronic and recurrent admission to hospital are linked with pus infected individuals (Kandemiret *al.,* 2007). Bacteria involved in pus formation are known as pyogenic,suppurative, or purulent but called mucopurulent when bacteria form mucus (Madiganet *al.,* 2006).

Staphylococcus aureus is associated with various skin and soft-tissue infections including folliculitis, impetigo, furuncles, carbuncles, hidradenitis suppurativa, and cellulitis (Wickreet *al.,* 1982). It can also cause toxic shock syndrome and staphylococcal scalded skin syndrome. (Ryan and Ray, 2004). Management includes removal of the focus of *S. aureus* (e.g., abscess drainage or tampon removal) and use of a betalactamase–resistant antistaphylococcal antibiotic in combination with clindamycin, which has the potential of reducing toxin production (Kaplan *et al.,* 2005). *Streptococcus pyogenes* is a beta-hemolytic bacterium causes a wide variety of diseases in humans (Schroederet *al.,* 2003). The bacterial toxins cause proteolysis of epidermal and sub epidermal layers, allowing the bacteria to spread quickly along the skin layers and thereby cause blisters or purulent lesions (Callister *et al.,* 2001). A ubiquitous organism, *S. pyogenes* is accounting for 15-30% in children and 5-10% in adults of the acute pharyngitis cases (Schroeder *et al.,* 2003).*E coli* infections include septic arthritis, endophthalmitis, suppurative thyroiditis, sinusitis, osteomyelitis, endocarditis, and skin and soft-tissue infections (especially in patients with diabetes) (Carpenter *et al.,* 1998). Patients with diabetes mellitus are also at high risk of developing pylephlebitis of the

portal vein and liver abscesses (Carpenter *et al.,* 1998).*Klebsiella pneumoniae*is an important constituent of intestinal flora (Ryan *et al.,* 2004).The most common infection caused by *Klebsiella*outside the hospital is pneumonia, typically in the form of bronchopneumonia and also bronchitis. These patients have an increased tendency to develop lung abscess, cavitationand empyema (Groopman et al., 2008).*Pseudomonas aeruginosa* being an opportunistic pathogen, most infections with this organism occur in immunocompromised hosts (Bendiak*et al.,* 2009).In immunocompromised hosts, including neonates, infection can progress rapidly through the 3 stages and cause pneumonia, endocarditis, peritonitis, meningitis, ecthymagangrenosum (EG), bacteremia, and overwhelming septicemia (Pollack, 1984).

Recent study was conducted to check the prevalence of the various gram positive and gram negative bacteria in pus samples isolated from the skin infection and their after consequences were determined by the antibiotics susceptibility pattern of the prevalent bacteria.Even withthe improvementsto control infections, problems of the multidrug resistancein wound infections have not completely prevented (Thomas, 1981). The extensive uses of antibiotics, followed with the length of time over which they have been available, have directed the resistant organisms to morbidity and mortality (Elmer *et al*., 1997). Awareness and understanding the causative agents of wound infection has demonstrated to be helpful in the selection of right antimicrobial therapy and use of appropriate measures taken in health institutions to control infection (Adebayor*et al*., 2003). Therefore, the present study aimed to evaluate the important causative agents of bacteria from pus samples and their antibiotic susceptibility patterns which may be imperative as it guides the selection of an effective regimen for the treatment.

Material and methods

This study was carried out in Microbiology Department of Sheikh Khalifa Bin Zayed Hospital Rawalakot Azad Kashmir, Pakistan. A total of 100 samples (54 and 46 from males and female respectively) were processed.

Sampling Process

Non-Probability Random sampling technique was followed. In inclusion criteria, all males and females were included, samples were taken from patients aged more than 10 years and admitted and outdoor both patients were also included in study. Whilepatients already

taking antibiotics**,** children and neonates aged less than 10 yearsand patients suffering from burn injuries were excluded. Sterilized cotton swabs were used for the specimen sampling from wounds.

Methods of preparation of agar media

Blood agar was prepared as instructed by the manufacturer. The Media was sterilized by autoclaving at 121C˚ for 15 minutes. Then it was transferred to a 50C˚ water bath. The blood was allowed to warm to room temperature before being added to the molten agar.The media was dispensed aseptically in sterile petri dishes. Date and batch number was mentioned on plates. Then plates were stored at 2–8 C˚ and sealed in plastic bags to prevent loss of moisture. Similarly, MacConkey's agar was prepared as instructed by the manufacturer, in 1 L of purified water and mixed thoroughly (Becton, Dickinson and company). Heated with frequent agitation and boiled for 1 minute to completely dissolve the powder. Autoclaved at 121°C for 15 minutes. Media was then dispensed aseptically in petri dishes. Date and batch number were mentioned on dishes.

Processing of Specimens

Sample of pus swabs were processed in the Microbiology Department of Sheikh Khalifa Bin Zayed Hospital. Pus swabs were directly streaked on Blood agar and MacConkey's agar medium. After inoculation these plates were incubated at 37° C for 18-24 hours.

Colony morphologyon cultured media

The following bacterial colonies were collected on the basis of their characteristic colony morphology on both Blood agar and MacConkey's agar medium as well as by confirmatory biochemical tests. The bacteria identified in this study were *S. aureus*, *Enterococcus faecalis*, *E. coli*, *Proteus vulgaris*, *Serratiamarcescens, Serratiaodorifera, Enterobacter cloacae, Acinetobacter baumannii*and *Pseudomonas aeruginosa.*

Antibiotics Susceptibility Test

The antimicrobial susceptibility test was performed using the modified disc diffusion test method (Kirby-Bauer) recommended by Clinical and Laboratory Standards Institute (CLSI). The following antimicrobial agents were used for Gram positive bacteria: ampicillin

(10ug), Erythromycin (15ug),Methicillin (5ug),Cotrimoxazole (25ug),Doxycyc:ine (30ug),Gentamycin (10ug),Amikacin (30ug),Vancomycin (10ug),Ciprofloxacin (5ug),Levofloxacin (10ug), Ceftriaxone (30ug),Moxifloxacin (5ug),Imipenem (10ug),Tazobactam/Pipracillin (10ug), Cefoperazone (75ug), Fusidic acid (10ug), Ceftazidime (30ug),Linezolid (30ug),and the following antimicrobial agents were used for Gram negative bacteria: Amplicillin (10ug), Amoxycillin (10ug), Ciprofloxacin (5ug), Gentamycin (10ug), Cefoperazone (30ug), Ceftazidime (30ug), Tazobactam/Pipracillin (10ug), Meropenem (10ug), Amikacin (30ug),

The Disk Diffusion Method (Kirby-Bauer)

Mueller-Hinton agar (MHA) media were prepared to carry out the antibiotic susceptibility pattern of each bacterial isolate by Kirby-Bauer's disc diffusion assay. Following the standard protocol of the manufacturer(38g agar in 1L distilled water), agar petri plates (20ml agar media/plate)were prepared after sterilization with autoclaving at 121ºC for 15 minutes at 15 psi. Petri plates were incubated at 37ºC overnight to check sterility.

Inoculum preparation

According to the protocol of Thornsberry and Swenson (1978) commercially available sterile distilled water ampoules were used for the preparation of inoculum. Inoculum was adjusted to 0.5 McFarland standards with pathogenic inocula. A sterile cotton swab was soaked by dipping into standardized bacterial suspension. To remove the excessive culture, swab was pressed with the inner walls of the tube. The inoculum on the swab was blowout uniformly all over the surface of Mueller–Hinton agar (MHA) smoothly in three directions to provide a uniform inoculum growth on the entire surface of the media (Pillay, 1999). The plates spread with inoculawere left to dry for 15 minutes and then with the help of syringe needle, discs of identified antibiotics were placed on the surface of inoculated plates. The plates were incubated at 35ºC for 18 hour in an inverted position. After 24 hours of overnight incubation, MHA plates were inspected and inhibition zones were measured for each antibiotic disc. The susceptibility patterns of bacterial isolates against antibiotics were analyzed according to CLSI guidelines (CLSI, 2015).

Results and Discussion

Bacterial isolates

*Staphylococcus aureus*can infect people of all ages without gender discrimination and was found the most frequent organism (49.35%) found in all pus samples. Seconds most abundant and common bacteria in pus samples found was *Pseudomonas aeruginosa (16.88%),* followed by *Acinetobacter baumannii (10.39%), Enterococcus faecalis (6.5%), Enterobacter cloacae (5.19%), Escherichia coli (3.9%), Proteus vulgaris (2.59%),Serratiamarcescens (2.59%)* and *Serratiaodorifera (2.59%)* as described in figure 1. *Staphylococcus aureus* sensitivity pattern agree with those reported by Taylor (1992) on surgical site infections. The results also justified with those of Buwembo (1990)) who identified *Staphylococcus aureus* as the commonest causative agent of potentially contaminated wounds in Mulago hospital.

Antimicrobial Susceptibility Pattern:

According to the report of the Centre for Disease Control and Prevention, antibiotics resistance in gram positive pathogen is a global and disastrous issue in which *S. aureus* and Enterococcus species are on front phase of the antibiotics resistance chart (CDC, 2013). In our study among the gram positive bacteria, the most resistant isolate was found, *Staphylococcus aureus* showed resistant to seven antibiotics with 55% average percent resistance and *Enterococcus faecalis*showed 24% average percent resistance to antibiotics (Tab.1.). Vancomycin was considered to be the drug of choice for *S. aureus* by Hansra and Shinkai (2011) but in our 31.58% of the *S. aureus* isolates showing resistance which is the sign of danger.

The susceptibility patterns of gram negative bacterial isolates to various antibiotics are shown in table 2 and 3 (resistance and sensitivity respectively).This study suggested that *Enterobacter cloacae* (67%) predominantly showed resistant to antibiotics followed by *Acinetobacter baumannii*(63.7%), *Escherichia coli* (56%), *Serratiaodorifera*, *Serratiamarcescens*(45%), *Pseudomonas aeruginosa* (41.5%) and *Proteus vulgaris* (30%). This correlates with a study carried out in hospitals of France at different periods describing that *Enterobacter cloacae* is the alarming and emerging antibiotic resistant bacteria which may poses serious health threats (Fig.2.).In our study it was observed that maximum isolates were sensitive to the Moxifloxacin antibiotic followed by Amikacin, Gentamycin, Meropenem, Doxycycline, Vancomycin, and Fusidic acid. The overall highest resistant rates were observed for most of the bacterial isolates (gram positive and gram negative) to

antibiotics Amplicillin, Cotrimoxazole and Amoxycillin followed by Ceftazidime, Cefoperazone, Methicillin and Tazobactam (Tab.1.). It was also reported that some ESBL-producing Enterobacteriaceae are resistant to nearly all antibiotics in the penicillin and cephalosporin classes (CDC, 2013). In this study the maximum gram negative isolates were sensitive to Cefoperazoneand Amikacin followed by Ciprofloxacin and Gentamycin (Tab.3.).

Conclusion

Formulation of an effective infection control policy is important in preventing skin infections. Simple procedures like hand washing are known to prevent cross contamination and must be strictly enforced. This will help in reducing the maximum numbers of infections. Antibiotics intake must be strictly regulated and there should be an appropriate hospital antibiotic policy to avoid misuse and overuse of antibiotics. This also reflected the crisis of the overuse of these antibiotics worldwide and lack of interest of the most pharmaceutical industries to develop new technologies to cope with this worstsituation.

Conflict of Interest Statement

It is declared by all the authors that there is NO conflict of interest or any kind of organization or financial involvement**.**

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Table 1: Percentage sensitivity pattern of different antibiotics againstgram positive bacteria

Antibiotics used	**Disk content**	***Staphylococcus Aureus* (n=38)**				***Enterococcus faecalis* (n=06)**			
		S	**%**	**R**	**%**	**S**	**%**	**R**	**%**
Amplicillin	(10ug)	0	0	38	100	1	16.67	5	83.33
Erythromycin	(15ug)	24	63.16	14	36.84	4	66.67	2	33.33
Methicillin	(5ug)	18	47.37	20	52.63	0	0	6	100
Cotrimoxazole	(25ug)	18	47.37	20	52.63	1	16.67	5	83.33
Doxycyc:ine	(30ug)	30	78.94	08	21.05	1	16.67	5	83.33
Gentamycin	(10ug)	23	60.52	14	36.84	4	66.67	2	33.33
Amikacin	(30ug)	25	71.42	13	34.21	0	0	6	100
Vancomycin	(10ug)	26	68.42	12	31.58	3	50	3	50
Ciprofloxacin	(5ug)	22	57.89	16	42.10	3	50	3	50
Levofloxacin	(10ug)	22	57.89	16	42.10	0	0	6	100
Ceftriaxone	(30ug)	24	63.16	14	36.84	1	16.67	5	83.33
Linezolid	(10ug)	25	71.42	13	34.21	1	16.67	5	83.33
Imipenem	(10ug)	18	47.37	20	52.63	0	0	6	100
Tazobactam/Pipracillin	(10ug)	15	39.47	23	60.53	0	0	6	100
Cefoperazone	(75ug)	17	44.73	21	55.27	0	0	6	100
Fusidic acid	(10ug)	25	71.42	13	34.21	1	16.67	5	83.33
Ceftazidime	(30ug)	09	23.68	29	76.32	0	0	6	100
Moxifloxacin	(5ug)	30	78.94	08	21.05	6	100	0	0
Average Percentage			55.00%		45%		24.07%		75.90%

(R = resistant, S = sensitive, % = percentage)

Table 2: Antibiotic susceptibility pattern (Percent resistant) of gram negative bacteria

Antibiotics used	**Disk content**	***Pseudomonas aeruginosa* (n=13)**		***Acinetobacter baumannii* (n=08)**		***Escherichia coli* (n=03)**		***Proteus vulgaris* (n=02)**		***Serratiaodorifera* (n=02)**		***Enterobacter cloacae* (n=04)**		***Serratiamarcescens* (n=02)**	
		R	%	R	%	R	%	R	%	R	%	R	%	R	%
Amplicillin	(10ug)	13	100	8	100	3	100	2	100	2	100	4	100	2	100
Amoxycillin	(10ug)	9	69.23	4	50	2	66.6	2	100	1	50	4	100	2	100
Ciprofloxacin	(5ug)	4	30.77	5	62.5	2	66.6	0	00	2	100	1	25	0	00
Gentamycin	(10ug)	1	7.69	5	62.5	0	00	0	00	0	00	3	00	1	50
Cotrimoxazole	(25ug)	9	69.23	5	62.5	2	66.6	1	50	2	100	4	100	2	100
Amikacin	(30ug)	1	7.69	4	50	0	00	0	00	0	00	3	75	0	00
Ceftazidime	(30ug)	11	84.62	5	62.5	3	100	1	50	2	100	4	100	2	100
Tazobactam	(10ug)	4	30.77	5	62.5	2	66.6	0	00	0	00	3	75	0	00

Meropenem	(10ug)	1	7.69	6	75	2	66.6	0	00	0	00	2	50	0	00
Cefoperazone	(30ug)	1	7.69	4	50	1	33.3	0	00	0	00	2	50	0	00
Average Percentage			41.5%		63.7%		56%		30%		45%		67%		45%

(R = resistant, S = sensitive, % = percentage)

Table 3: Antibiotic susceptibility pattern (Percent sensitive) of gram negative bacteria

Antibiotics	**Disk content**	***Pseudomonas aeruginosa* (n=13)**		***Acinetobacter baumannii* (n=08)**		***Escherichia coli* (n=03)**		***Proteus vulgaris* (n=02)**		***Serratiaodorifera* (n=02)**		***Enterobacter cloacae* (n=04)**		***Serratia marcescens* (n=02)**	
		S	**%**	**S**	**%**	**S**	**%**	**S**	**%**	**S**	**%**	**S**	**%**	**S**	**%**
Amplicillin	(10ug)	0	0	0	00	0	00	0	00	0	00	0	00	0	00
Amoxycillin	(10ug)	4	30.77	4	50	1	33.3	0	00	1	50	0	00	0	00
Ciprofloxacin	(5ug)	9	69.23	3	37.5	1	33.3	2	100	0	00	3	75	2	100
Gentamycin	(10ug)	12	92.31	3	37.5	3	100	2	100	2	100	1	25	1	50
Cotrimoxazole	(25ug)	3	23.08	3	37.5	1	33.3	1	50	0	00	0	00	0	00
Amikacin	(30ug)	12	92.31	4	50	3	100	2	100	2	100	1	25	2	100
Ceftazidime	(30ug)	2	15.38	3	37.5	0	00	1	50	0	00	0	00	0	00
Tazobactam	(10ug)	9	69.23	3	37.5	1	33.3	2	100	2	100	1	25	2	100
Meropenem	(10ug)	12	92.31	2	25	1	33.3	2	100	2	100	2	50	2	100
Cefoperazone	(30ug)	12	92.31	4	50	2	66.6	2	100	2	100	2	50	2	100
Average Percentage			57%		36.2%		43.3%		70%		55%		25%		55%

(R = resistant, S = sensitive, % = percentage)

Figure 1: Over all Percentage (%) of growth positive samples.

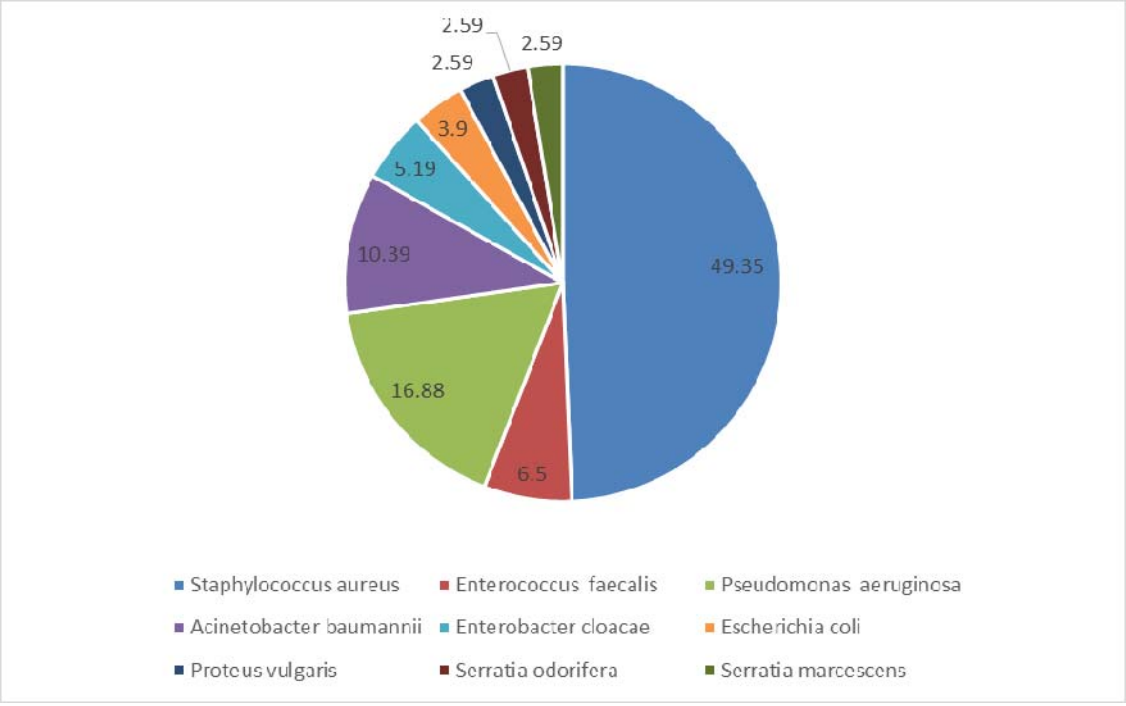
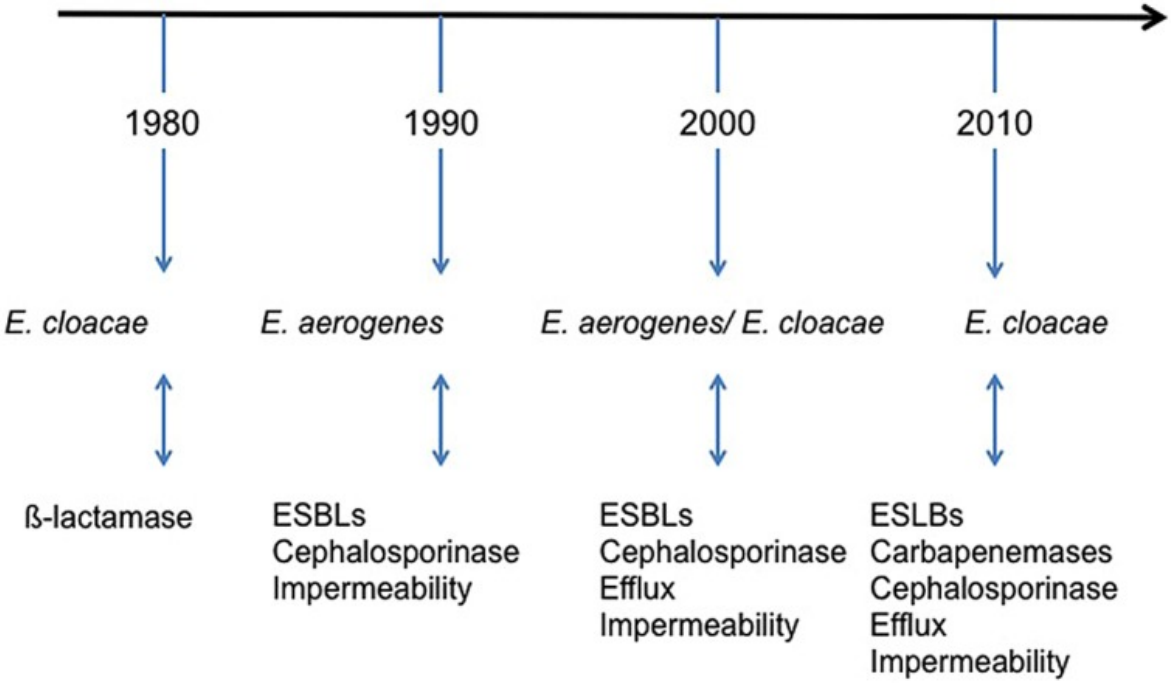


Figure 2: Showing timing emergence of the antibiotic resistance bacteria (Arpin et al., 1996; Bornet et al., 2000; Chevalier et al., 2008; Lavigne et al., 2012; Mezzatesta et al., 2012; Anastay et al., 2013; Robert et al., 2014).



ESBLs, extended-spectrum β-lactamases.