

## Isolation and identification of some uncommon bacterial species isolated from different clinical sample

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### Abstract

There are many opportunistic bacterial species that are uncommon and infrequently exist in clinical specimen, most of them are difficult to routine identification, even some of them are poorly documented in clinical specimen. also had no less role in the coordinates of the disease than common bacterial species. Six hundred and fifty samples were collected from patients attending to some hospitals in Sulaimanya City and Kalar General Hospital during the period from October 2015 to November 2016.

Samples were firstly cultured on different media in order to isolate and identify bacterial isolates according to cultural characteristics, morphological features and biochemical reactions in addition to Vitek 2 system for identifying uncommon and infrequent isolates. The identification and susceptibility test were performed in Kalar General Hospital. Isolated 286(44%) bacterial strains from different clinical samples, 125 of them were identified by Vitek 2 automated system, while 23(8%) of isolates were considered as uncommon bacterial species.

The antimicrobial susceptibility of uncommon isolates, showed significant variation against twenty four antibiotics. Four isolates; *Acinetobacter baumannii*, *Acinetobacter calcoaceticus*, *Enterobacter ludwigii* and *Gemella sanguinis* were resistant to all antibiotics. Whereas *Aerococcus urinae*, (*Citrobacter freundii* and *Alloiococcus otitis*), (*Morganella morganii*, *Sphingomonas paucimobilis*, *Pantoea agglomerans*, *Streptococcus sobrinus* and *Kocuria rosea* n.2), (*Ochrobactrum anthropi* n.1 and *Kocuria kristinae*), (*Ochrobactrum anthropi* n.2 and *Kocuria rosea* n.1), (*Pseudomonas stutzeri*, *Pseudomonas fluorescens* and *Citrobacter koseri*), (*Aeromonas salmonicida* and *Micrococcus luteus* n.1) and *Micrococcus luteus* (n.2) resistant to the antibiotics as these percentages 95.8%, 91.6%, 87.5%, 83.3%, 79.1%, 70.8%, 66.6% and 62.5%, respectively.

## Introduction

Coagulase negative staphylococci are the most common microorganisms isolated from clinical specimen, however, 85% of the isolates are contaminants, usually from skin contamination at the time of collection (Weinstein *et al.*, 1997), also *Escherichia coli* (Bodey *et al.*, 1986), *Klebsiella pneumoniae* (Bodey *et al.*, 1989), *Pseudomonas aeruginosa* (Bodey and Jadeja 1985), *Staphylococcus aureus*, *Streptococcus pneumoniae* (Folland *et al.*, 1974) considered as the most common bacterial species pathogens from human clinical sites. Citrobacter species is the best example of these bacterial species that are infrequent nosocomial pathogens and difficult in routine identification, usually utilize citrate as a sole carbon source. In addition to Citrobacter spp. there are some bacterial species that are uncommon or infrequently exist in clinical sample such as *Acinetobacter calcoaceticus* (Pal and Kale 1981), *Pseudomonas stutzeri* (Robert and Overman 1994), *Pseudomonas fluorescens* (Guttman 2008), *Sphingomonas paucimobilis* (Bhatia and Tomar 2016), *Kocuria kristinae* (Tewari *et al.*, 2013 and Chen *et al.*, 2015), *Gemella sanguinis* (Ma *et al.*, 2015).

The identification by Vitek 2 system within 3 hours using fluorescence reading, the weakness of this system was the breadth of its identification database, especially for *Citrobacter* spp., *Enterobacter* spp., *Pantoea* spp., nonfermenting bacilli, such as *Pseudomonas* spp. and *Acinetobacter*, and for gram-positive cocci, such as Streptococcaceae (Jossart and Courcol 1999). Here we aims in this study; isolation and identification of some species of opportunistic bacteria that are uncommon and difficult to investigation in routine laboratory method, in addition to determine resistance of their uncommon isolates during antibiotic susceptibility.

## Materials and methods

### Specimen collection

Six hundred and fifty samples were collected from different clinical specimens including 496 urine, 71 stool, 34 blood, 33 wound, 16 sputum from Kalar General Hospital and some hospital Lab. in Sulaimanya City, during the periods from October 2015 to November 2017.

### Isolation of bacterial species

Patients samples were transferred to the laboratory, then inoculated onto blood, MacConkey and mannitol salt agar by streaking method and incubated at 37°C for 18-24 hours (if no growth on plates reincubated for 48 hours); single colonies that produce pigment or any characteristic

were transferred to nutrient agar and subcultured more than one time to obtain pure cultures( Levinson 2010).

### **Maintenance and storage of isolates**

To preserve the diagnosed bacterial isolates, without losing their genetic characteristics, nutrient agar plate was streaked with a single colony of bacteria, after 24 hours appearance of growth at 37°C, one ml of nutrient broth was added to the surface of each plate and the growth harvested, then transferred to small vials containing nutrient broth with sterilized 1ml of 20% glycerol, then stored at -20°C for several months as long term stock culture. Also nutrient agar slants were stored during the work in refrigerator as worked stock culture for less than a month (Ausubel *et al.*, 1987).

### **Identification of bacterial species**

Microscopic examination of stained bacteria by gram stain was done, which is one of the initial step towards a presumptive identification, also oxidase, catalase test and cultural characteristics on selective media were used as presumptive identification.

In addition to routine identification, we used Vitek 2 automated system; A bacterial suspension was made by sterile saline and made only one dilution for each test as simplicity, reading the barcode as traceability before introducing the bacterial suspension into the cassette, after 70 second introducing the cassette into the sealer chamber ( the sealing card and introduction into the reader-incubater was done by automated system). Automated injection of the cards regards the end of analysis cited by manufacture company (Biomerieux).

### **Antibiotic susceptibility test**

It was done by disc diffusion method, eight discs spaced evenly, approximately 15mm from the edge of the plate, and one disc placed in the center of the plate. Each disc was gently pressed down to ensure even contact with the medium. The plates were incubated at 37°C overnight. The size of inhibition zone was measured by ruler on the under surface of the plate without opening the lid using the method described by Kirby-Bauer cited by(Vandepitte *et al.*, 2003), and the result was compared with the standard diameter of inhibition zones for each antibiotic according to (CLSI). The twenty four antibiotic discs used in this research were Meropenem, Dorepenm, Azteronam, Pencillin G, Pipracillin, Ampicillin, Amoxiclave, Cefotaxime,

Ceftriaxon, Cefazolin, Cephalothin, Cefadroxil, Cefexime, Cefepem, Ceftazidime, Ciprofloxacin, Levofloxacin, Amikacin, Netilmicin, Tobramycin, Nitrofurantion, Doxycycline, Tetracycline and Trimethoprim.

### Result and discussion

From more than 650 clinical sample in different hospitals in Sulaimanya and Kalar city during the period from October 2015 to November 2016. Out of 286(44%) bacterial strains were isolated, 125 of them were identified by Vitek 2 system. About 260(90%) of isolates were common bacterial species and 23(9.8%) of isolates were considered as uncommon bacterial species, whereas 3(1%) of isolates were not identified by Viteck 2 system as shown in table(1) and table(2).

**Table(1):** number of uncommon bacteria isolates.

Uncommon bacterial isolates	No. of isolates	Sample isolates
<i>Citrobacter freundii</i>	1(0.34%)	urine
<i>Citrobacter koseri</i>	1(0.34%)	urine
<i>Acinobacter calcoaceticus</i>	1(0.34%)	urine
<i>Acinobacter baumannii</i>	1(0.34%)	sputum
<i>Sphengomonas paucimobilis</i>	1(0.34%)	urine
<i>Morganella morgani</i>	1(0.34%)	urine
<i>Enterobacter ludwigii</i>	1(0.34%)	urine
<i>Pseudomonas fluorescens</i>	1(0.34%)	sputum
<i>Aeromonas salmonicida</i>	1(0.34%)	sputum
<i>Pantoea agglomerans</i>	1(0.34%)	urine
<i>Ochrobactrum anthropi</i>	2(0.68%)	urine
<i>Pseudomonas stutzeri</i>	2(0.68%)	urine
<i>Kocuria rosea</i>	2(0.68%)	Blood, urine
<i>Kocuria kristinae</i>	1(0.34%)	urine

<i>Mircoccus luteus</i>	2(0.68%)	urine
<i>Aerococcus urinae</i>	1(0.34%)	urine
<i>Alloiococcus otitis</i>	1(0.34%)	urine
<i>Gemella sanguinis</i>	1(0.34%)	urine
<i>Streptococcus sobrinus</i>	1(0.34%)	urine

**Table(2):**Total number of bacterial isolates.

Bacterial isolates	No. of isolates
<i>E. coli</i>	148(51.7%)
<i>Klebseilla spp.</i>	37(12.9%)
<i>Pseudomonas aeruginosa</i>	16(5.5%)
<i>Staphylococcus aureus</i>	15(5.2%)
<i>Proteus spp.</i>	14(4.8%)
<i>Staphylococcus hemolyticus</i>	8(2.7%)
<i>Staphylococcus hominis</i>	5(1.7%)
<i>Staphylococcus saprophyticus</i>	5(1.7%)
<i>Staphylococcus lentus</i>	3(1.04%)
<i>Staphylococcus xylosus</i>	3(1.04%)
<i>Staphylococcus epidermidis</i>	3(1.04%)
<i>Enterococcus faecalis</i>	3(1.04%)
Uncommon bacterial species	23(9.8%)
Unidentified bacterial isolates	3(1.04%)
Total isolates	288

In a study in Switzerland by Gayral *et al.*, (1997), they were evaluated Vitek 2 system for rapid identification of the 845 strains medically relevant gram negative rod. Only 7 (0.8%) strains were misidentified, and 10 (1.2%) were not identified. Thus its database was challenged by a diverse group of organisms, including species which are very rarely encountered in the routine clinical laboratory.

This result is near to a study by Rit *et al.*, (2013) they were isolated one strain of *P. stutzeri* from urine sample when collected 201 different isolates of nonfermentor gram negative from

various clinical sample. Also in a study in USA by Helio *et al.*, (2005) they were isolated *O. anthropi*, *S. paucimobilis*, *P. stutzeri*, *P. fluorescens/P. putida* and *Aeromonas* spp.: 0.8, 0.8, 1.1, 7.2 and 11.0 in different clinical samples, respectively when they collected 3059 strains of non-enteric gram negative bacilli as part of the Sentry Antimicrobial Surveillance Program during (1997–2003).

Studies concerning the prevalence of *A. otitis* in otitis medea have been performed only in Finland (Hendolin *et al.*, 1997 and Leskinen *et al.*, 2002) and in the United Kingdom (Beswick *et al.*, 1999). Other than in these two countries, only a few clinical strains of *A. otitis* have been isolated in the United States (Faden and Dryja. 1989), Turkey (Kalcioğlu *et al.*, 2002), Spain (Gomez-Hernando *et al.*, 1999), and Brazil (Sih *et al.*, 1992). In Asian countries, even the isolation of *A. otitis* has not been reported yet.

All isolates were growth on MacConky's plate considered as gram negative rods with observed by gram staining which is mentioned by Forbes *et al.*, (2007), in addition to the ability of lactose fermenting. Oxidase and IMViC test were the best separation for differentiation infrequent gram negative bacterial species from common species. as well as the bacterial growth on blood agar and nutrient agar or one of them without growth on MacConky's agar were considered as gram positive cocci by gram staining which is also mentioned by Forbes *et al.*, (2007), in addition to the ability of hemolysis. Catalase test and growth on Manitol sult agar after subculturing were the best separation between Staphylococci and Streptococci. All reactions were recorded daily, with final readings at 72 hr. No changes in reactions were observed past 72 hr.

The production of methyl red and citrate utilizing were the strong parameter for identifying as *Citrobacter* spp. in addition to the ability to lactose fermenting which was pink in *C. freundii* as the same reaction in *E. coli*, whereas it was weaken in *C. koseri*. There are many studies that have been conducted on citrobacter by Raof *et al.*, (2013) in Tikrit, that they are disagree with present result because they were depended on routine identification which is not efficient to citrobacter identification. Whereas there are many studies near to present result by Al-Hissnaway *et al.*, (2010) in Iraq and (Shahid 2010) in india.

The colonies characteristic of *Acinetobacter calcoaceticus* and *Acinetobacter baumannii* were as follows: On MacConkey's agar the colonies were pale and non-lactose fermenting. The result of

gram staining showed gram negative coccobacilli it is important characteristic with negatively oxidase in routine identification.

In a multicentre study by the Centers of Disease Control(CDC), Atlanta, Georgia, over a period of four years *A. calcoaceticus* was the causative organism in 1372 of 180 982 cases of infections reported to the center (Gajaylife 1984). Kaplan *et al.*, (1984) reported a case of postoperative urinary tract infection and also attempted the detection of humeral antibody against the organism.

The characteristics of *S. paucimobilis* was gram negative bacilli and had yellow-pigmented and nonmucoid colonies on nutrient agar plates that were positive. Colonies on MacConky's were non-fermented lactose. Kilic *et al.*, (2007) concluded that *S. paucimobilis* strains can cause outbreaks in hemato/oncology units. They did not demonstrate genetic relatedness between clinical and environmental isolates by pulsed-field gel electrophoresis (PFGE).

The characteristics of *Ochrobactrum anthropi*, colonies were as follows: On nutrient agar, there were small size, circular, low convex, smooth, shining with entire margin, and non-hemolytic colonies on blood agar. Non lactose fermenting isolate was obtained after overnight incubation on MacConkey's agar.

Theodore *et al.*, (1992), who described the first patient infected by *O. anthropi* in a child, that of bacteremia in a 3-year-old girl undergoing chemotherapy for retinoblastoma. also they absorbed that *O. anthropi* closely related bacterial species, namely *Alcaligenes xylosoxidans* subspecies *xylosoxidans*, *Agrobacterium radiobacter*, and "*Achromobacter*" group B.

The colony morphology of *M. morgani* isolates on MacConkey agar appears large, smooth, convex, and translucent as well as it was non lactose fermenting, also it was a gram negative coccobacilli in gram staining smear.

This study were near to local study in Bagdad by Auda J. Gh. and Al-Grawi (2009) who was isolated two strain form 220 urine sample during two years and with global by Friedman *et al.*, 2015 in Tel Aviv who isolated one strain of *M. morgani* from 257 children initially diagnosed with UTI. While there were studies recognized most commonly in hospitalized with indwelling urinary catheters and in surgical (Gates *et al.*, 1986).

The colonies characteristic of *A. salmonicida* were circular, entire, raised, glistening, cream colored on nutrient agar and odor producing. No pigment was produced at either 22°C or 37°C

after growth for 1 week. Gram staining appeared some description about size and shape, this phenomenon maybe the first time in this species.

*A. salmonicida* is considered to be nonpathogenic for humans as it cannot grow at 37°C. “However, In our laboratory culture plates and broths were incubated twice at 37°C and each time the same types of colonies were isolated which were identified as *A. salmonicida* by Vitek 2 compact system. Based on the previous studies, *A. salmonicida* that non pigmented and will able growth at 37°C was atypical strain and the reliability of using pigment production in diagnosing typical *A. salmonicida* has also previously been questioned (Austin & Austin 1987).

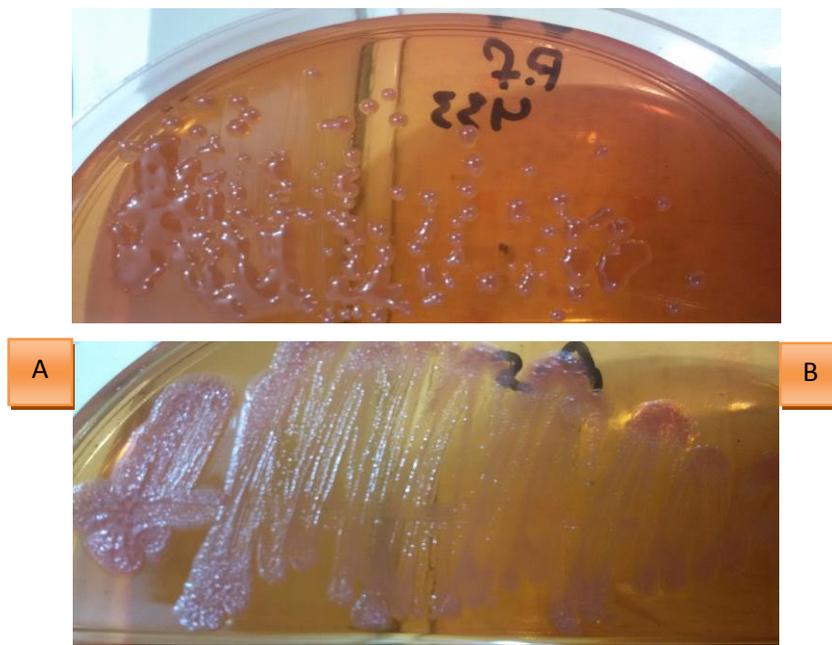
*E. ludwigii* was identified based on Vitek 2 (BioMerieux, France) it was very good identification as confidence, in spite of Vitek 2 can be separated their result to related species. *E. ludwigii* was the accurate species up on separated tests that suggested from the Vitek report.

In a Doctoral study in Nottingham by Abdulla (2014) who isolated one strain of *E. ludwigii* from neonatal meningitis when she was identified by 16S rRNA. also Hoffmann *et al.*, (2005) conducted *E. ludwigii* strain belongs to the *E. cloacae* complex was first reported and nominated in 2005 and later been found in many hospitals.

*P. fluorescens* was isolated from sputum in a patient suspected with tuberculosis. The growth appeared after 48 hours with mucoid colonies. This mucoidly may be due to pigment production that was yellow-green fluorescent as shown in below figure.

Colonies of *P. stutzeri* on nutrient agar were dry, coherent, ridged (rough)with concentric spreading areas and non-pigmented as shown in below figure. They were gram negative rod which was seen in single arrangement. Both strains grew as non-lactose fermenting colonies on MacConkey's agar. Fresh colony that was picked off from nutrient agar gave positive reaction for oxidase.

In a local study in Thi-Qar by Abas *et al.*, (2014) on detection on multidrug resistant of *Pseudomonas* spp. , isolated one strain of *P. fluorescens* from wound swab when collected 210 clinical sample. Although in a study by Shrooq *et al.*, (2014) in Bagdad city on the ability of *P. fluorescens* to produce bacteriocin when they observed inhibition of some gram negative and positive.



**Figure:** A. Showing mucoid colonies of *P. fluorescens* on MacConkey agar; B. Showing dry colonies of *P. stutzeri* on MacConkey's agar.

There are two main types of colony of *P. stutzeri* observed on nutrient agar by Lapagel (1968), they described the first type as the same our type (rough) and the second type, they described as a mucoid colony ("smooth"). Whereas study of Stanier *et al.*, (1966) they were founded both colony forms (Rough and smooth) when isolated from human sources.

The colonies of *M. luteus* were convex, smooth, and yellowish on blood and nutrient agar. The organism was positive for catalase and oxidase, as well as it produced yellow pigment that was considered as identification criteria. Arrangement were tetrad in the gram staining and the confidence identification Vitek was excellent.

Peces *et al.*, 1997 concluded that *M. luteus* should be considered as an emerging nosocomial pathogen in immunocompromized patients. Persistent bacteraemia unresponsive to medical management should be treated by catheter removal.

Colonies of *K. rosea* were circular, slightly convex, smooth (occasionally rough) on nutrient and blood agar. Colony morphology and color become more distinct with age. Bacterial cells were spherical and occurred in pairs, tetrads, and clusters. All biochemical tests in urine strain were negative only POLYB was positive in the Vitek biochemical profile and the probability identification was 89%. But we observed only two enzymes in the biochemical profile in blood strain; also the probability was 94%.

*K. kristinae* was grown on blood agar and nutrient agar, whereas no growth was observed on McConkey's agar. Gram staining of the colonies revealed the presence of gram positive cocci which were mostly arranged in tetrads. The organism which was catalase positive. They possess positive reaction for some sugar such as glucose (AGLU), maltose (dMAL), (SAC), (LAC), and optochin (OPTO) in the Vitek biochemical profile and the probability identification was 89%. These distinctive characteristics were unique in this strain among micrococci which means it is able to utilize these sugars as well as that is able to ferment lactose, in addition to resist against optochin antibiotic.

In the recent reported cases by Tewari *et al.*, (2013) of *K. kristinae* in urinary tract infection in a catheterized, 20-years old male that was considered as the first reported case of a catheter related urinary tract infection which was caused by *K. kristinae*. In another report in India by Lakshmikantha *et al.*, (2015) they isolated *K. kristinae* from urine and blood samples.

We believe that a reliable identification of *Kocuria* spp. is possible with the use of the Vitek 2 GP card. Since these isolates are rare in the routine microbiology laboratory. A better understanding of the potential pathogenicity of this species will be more apparent after the report of more cases and possess ingredients of resistance to antibiotics.

The growth of *A. urinae* was observed on blood agar and nutrient agar with appeared  $\alpha$ -hemolytic and small colonies. As well as the gram staining showed single cell, diplococci, streptococci and tetrad in arrangement. A negative catalase reaction and positive reaction of optochin was showed in Vitek result and the probability was 89%.

*A. urinae*, previously known as *Aerococcus*-like organism, is an uncommon pathogen. Previous reports from European countries indicate that it is associated with UTI (Christensen *et al.*, 1991), bacteremia (Christensen *et al.*, 1995).

Our result of the isolation of *A. urinae* was near to that of a study in USA by Sierra-Hoffman *et al.*, (2005) that revealed the rate of *A. urinae* recovery 0.25% in the study period during (2001-2002) who receives from microbiology laboratory approximately 30000 specimens per year for urine culture.

The isolation of *A. otitis* in urine sample was a first time and was grown slowly on blood and nutrient agar, Only PyrA and dTRE were positive reaction in biochemical Vitek profile and the probability was 93%. It has been recovered from the middle ear of children with acute otitis

media, studies showed that *A. otitis* was frequently found in the outer ear canals of healthy volunteers (Frank *et al.*, 2003). It is very difficult to culture. So far, only a few clinical strains of *A. otitis* has been isolated (Faden and Dryja 1989). However, previous studies by Hendolin *et al.*, (1997) showed that *A. otitis* was never detected by culture, but it was detected by PCR more frequently than other middle ear pathogens.

Colonies Of *G. sanguinis* were grown on blood agar plates after 48 hour of incubation. The colonies after 24 hours appeared small, circular, entire, translucent, smooth, and non-pigmented. Catalase and oxidase reaction were negative. According to the Vitek biochemical profile, only three reactions were positive among 43 tests, namely; APPA, OPTO and PHOS. Although the probability identification was 97%.

This result is near to a study by Ma *et al.*, (2015) they observed the colonial growth on blood agar after 48 hr. of incubation, which showed non-hemolytic, tiny, pinpoint, smooth, translucent to grayish colonies. Gram stain showed gram-positive cocci in pairs. Also it was identified as *G. sanguinis* by Vitek 2-Compact System (Biomérieux, France).

We have been found that *A. calcoaceticus*, *A. baumannii*, and *E. ludwigii* were resistant to all antibiotics. Whereas *C. freundii* isolates were resistant to twenty two antibiotics 91.6%, and it was sensitive for Meropenem and Doripenem.

Although *S. paucimobilis*, *M. morgani* and *P. agglomerans* were resistant to twenty one antibiotics 87.5%, but they were sensitive for (Meropenem and Doripenem and Pepracillin), (Meropenem, Levofloxacin and Ciprofloxacin) and (Meropenem, Cefexim and Netilmicin), respectively.

*O. anthropi* (n.1) was resistant to twenty antibiotics 83.3%, and it was sensitive for Meropenem, Doripenem, Levofloxacin and Ciprofloxacin but another strain (n.2) was resistant to nineteen antibiotics 79.1%, and it was sensitive for Meropenem, Doripenem, Levofloxacin and Ciprofloxacin in addition to Doxycycline.

Although *C. koseri*, *P. stutzeri*(n.1 and n.2) and *P. fluorescens* were resistant to seventeen antibiotics 66.6%, whereas they were sensitive for Meropenem, Doripenem, Levofloxacin and Ciprofloxacin, in addition to Tobramycin, Amikacin and Netilmicin, but the second strain of *P. stutzeri*(n.2) was sensitive to the same antibiotics except Cefepem instead of Amikacin.

Finally, *A. salmonicidis* as the more sensitive strain among gram negative that were resistant to the sixteen antibiotics 62.5%, and it was sensitive for Meropenem, Doripenem, Azteronam, Cefepem, Ciproflxacin, Doxycycline, Tetracycline and Nitrofurantion.

In the light of above results, all of these bacterial species, possess ingredients of resistance in different degrees because each bacterial species possesses enzyme or some enzymes, structure or more and intrinsic resistance.

*A. baumannii*, *A. calcoaceticus* and *E. ludwigii* have possessed all ingredients of resistance, that are acquired in the host when exposed to drugs and host immune system or possessed normally. Whereas *C. freundii* has possessed all ingredients of resistance except to one group of  $\beta$ -lactam that is carbapenem which inhibits activity of bacterial enzymes that is essential for peptidoglycan synthesis (penicillin binding proteins), in addition to inhibition by carbapenem it was inhibited by Tetracycline that's tetracyclines group which inhibits protein synthesis (Kiser *et al.*, 2011).

Also *S. paucimobilis* was inhibited by carbapenem group and Piperacillin that is penicillin group of  $\beta$ -lactam which is the same action of carbaenem but *M. morgani* was inhibited by carbapenem group and Ciprofloxacin that is fluoroquinolones group which inhibits DNA gyrase in gram negative bacteria. (Kiser *et al.*, 2011).

Both strains of *O. anthropi* have been inhibited by carbapenem and fluoroquinolones group but the second strain has been inhibited by carbapenem and fluoroquinolones in addition to Doxycycline that's tetracyclines group. They are bacteriostatic and so are used less often because of the bactericidal effect of the  $\beta$ -lactams. (Kiser *et al.*, 2011).

Although *C. koseri*, *P. stutzeri* (n.1) and *P. fluprescens* has been inhibited by carbapenem and fluoroquinolones in addition to Tobramycin, Netilmycin and Amikacin that are aminoglycosides which is preventing translation of mRNA during binds to the 30S ribosomal subunit (Kiser *et al.*, 2011), also another strain of *P. stutzeri* (n.2) was susceptible to the same antibiotics in addition to Cefepem which is fourth generation of cephalosporins and acts as natural penicillins.

Whereas *A. salmonicida* was susceptible to a wide range of antibiotics, some of them  $\beta$ -lactams such as, carbapenem , Cefepem, Azteronam which act as natural penicillins, and others such as, aminoglycoside, tetracyclines, Ciprofloxacin and Nitrofurantion act on damaging bacterial DNA . Macrobid Drug Label FDA. Retrieved 21 April 2014

Only one gram positive isolates that was resistant to all antibiotics or possessed all ingredients of resistance as we discussed in susceptibility in gram negative. However, that gram positive may be more resistance due to its possession the thick cell wall which protects the cell from toxic substances. *G. sanguinis* was resistant to all antibiotics, whereas *Aerococcus urinae* was resistant to twenty three antibiotics (95.8%) except Nitrofurantion that acts on damaging DNA. Also *All. otitis* was resistant to twenty two antibiotics 91.6%, and it was sensitive for Nitrofurantion and Netilmicin as well as that *S. sobrinus* was resistant to twenty one antibiotics 87.5%, and sensitive for Meropenem, Pepracillin and Nitrofurantion.

Whereas Micrococcaceae have less resistance, when *K. kristinae* was resistant to twenty antibiotics 83.3%, and susceptible to  $\beta$ -lactam; carbapenem group, Cefadroxil and Cefazolin that are the first generation of cephalosporins which are act as natural penicillins (Kiser *et al.*, 2011).

Both strains of *K. rosea* are different to susceptibility. *K. rosea* (n.2) was isolated from urine sample that was more resistance than the another strain, then each of *K. rosea* (n.2) and *S. sobrinus* were resistant to twenty one antibiotics 87.5%, whereas *K. rosea* (n.2) was sensitive for Pipracillin, Ampicillin and Nitrofurantion, also *S. sobrinus* was sensitive for each Pipracillin, Meropenem and Nitrofurantion. But another strain of *K. rosea*(n.1) that isolated from blood sample was resistant to nineteen antibiotics 79.1%, and it was susceptible to carbapenem group, fluoroquinolones and Tetracycline.

Either *M. luteus*, both strains are more sensitive to a wide range of antibiotics in composition to others. *M. luteus* (n.1) was resistant to sixteen antibiotics 66.6% , and it was susceptible to the followings; carbapenem group, Pepracillin, Cefazolin, cefepem, fluoroquinolones and tetracyclines. Whereas another strain (n.2) was resistant to fifteen antibiotics 62.5%, and it was susceptible to the followings; carbapenem group, Pepracillin, Cefazolin, Ampicillin, Ceftriaxone, Ciprofloxacin, Netilmicin and tetracyclines.

That was a report on 219 strains of *Kocuria* and *Micrococcus* showed that the majority of strains were sensitive to doxycycline, ceftriaxone, cefuroxime, amikacin, and amoxicillin with clavulanic acid, but resistant to ampicillin and erythromycin (Szczerba 2003).

The high sensitivity of *M. luteus* to  $\beta$ -lactam antibiotics may result from the presence of a reduced set of penicillin-binding proteins and the absence of a *wblC* gene, which plays an important role in the antibiotic resistance in other actinobacteria( Young *et al.*, 2010).

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