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Emerging of Yokenella regensburgei as Uropathogen: First Report

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Abstract
The study aimed to evaluate the prevalence of *Yokenella regensburgei* and to determine the phenotypic resistance patterns of this bacterium.A total of 300 urine samples were collected during a period intervals from February till July of the year 2013 from patients complained from urinary tract infections visited An- Najaf province Hospitals. The samples in all had been cultured on MacConkey agar where the results revealed that there were 250 of isolates lactose fermentative versus to 50 were lactose non fermentative .The lactose non fementative isolates then subjected to further identification and the phenotypic resistance patterns by Vitek-2 system had been conducted. The results revealed as first report identification of *Yokenella regensburgei* as agent of urinary tract infection with occurrence rate of 0.33% which might indicated the risk of related underlying diseases . the results of antibiotic susceptibility showed that the bacterium was sensitive to most screened antibiotics beside nitrofurantoin, tetracycline and trimethoprim/sulfamethoxazole shown activity against the bacterium.
Keywords: *Yokenella regensburgei* , Vitek 2, antibiotic susceptibility

1. Introduction

Yokenella regensburgei is one of a number of rarely encountered members of the family Enterobacteriaceae that infrequently been isolated from humans. Formerly this bacterium identified as NIH biogroup 9 by the National Institutes of Health in Japan (Kosako *et al.,* 1984) also as enteric group 45 by the Centers for Disease Control and Prevention (Hickman-Brenner *et al.,*1985), biochemically it greatly resembles *Hafnia alvei*. Kosako *et al.* (1984) suggested the name *Y. regensburgei* for this new genus and species while the Centers for Disease Control and Prevention proposed the name "*Koserella trabulsii*" for enteric group 45 (Hickman-Brenner *et al.,*1985).So it was recognized that *Y. regensburgei* and "*K. trabulsii*" are objective synonyms (Kosako *et al.,* 1987) and Kosako and Sakazaki (1991) proposed that the name *Y. regensburgei* had priority over "*K trabulsii*" upon the basis of rule of the Bacteriological Code. In 1991, the Centers for Disease Control and Prevention approved that *Y. regensburgei* had priority upon the basis of prior publication and has since dropped the use of the name "*K. trabulsii*" (McWhorter *et al*.,1991). *Yokenella regensburgei* is an opportunistic human pathogen and is the only species of the genus Yokenella within the family Enterobacteriaceae (Stock *et al*., 2004; Abbott and Janda, 1994). *Y. regensburgei* has been recovered from the intestinal tracts of insects, well water, and a number of anatomic sites in humans, including wounds of the limbs, the upper respiratory tract, urine, feces, and knee fluid (Hickman-Brenner *et al.,*1985, Kosako *et al.,* 1984).

2. Materials and methods

2.1. Collection of samples

The urine samples from patients complained from urinary tract infections were collected aseptically then these samples transfered as soon as possible to laboratory to avoid contamination, the samples subjected to different culturing and biochemical tests.

2.2. Isolation and Identification

The identified isolates which had been cultured on Macconky agar, MacConkey's agar medium is specially made to distinguish lactose non-fermenting (pale or colorless colonies) from lactose fermenting bacteria (pink to red colonies) furthermore the identification to species level confirmed by using automated Vitek 2 system which was briefly described. Suspension was prepared according to the manufacturer's recommendations of bioMérieux company by transferring sufficient number of colonies from overnight pure culture by swab and suspending the microorganism in 3.0 ml of sterile saline in a can tube . Turbidity had been adjusted to be from 0.5 to 0.63 using a turbidity meter called DensiChek. The same suspension was used in identification and antibiogram testing with VITEK-2 compact system. The identified. isolates were confirmed with the automated VITEK-2 compact system by using GN-ID cards (BioMérieux Company). This can briefly described as folllowing:

GN-ID cards were inoculated with microorganism suspensions using an integrated vacuum apparatus. A test tube containing the microorganism suspension was placed into a special rack (cassette) and the identification card is placed in the neighboring slot while inserting the transfer tube into the corresponding

suspension tube.The cassette can accommodate up to 10 tests with VITEK-2 compact system. The filled cassette was placed manually into a vacuum chamber station. After the vacuum was applied and air was re-introduced into the station, the organism suspension was forced through the transfer tube into micro-channels that fill all the test wells.

2.3. Minimum Inhibitory Concentration (MIC) by Vitek system.

Antibiotic susceptibility testing was performed with the automated VITEK-2 compact system based on MIC technique determination by using AST-N82 cards. This card contained the following antibiotics, Ampicillin, Amoxicillin/ clavulanic acid, Ampicillin/ sulbactam , piperacillin/tazobactam, cefazolin, cefepime, ceftazidime,ceftriaxone, aztreonam, imipenem, meropenem, amikacin, tobramycin, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, tigecycline, nitrofurantoin.

AST-N82 cards were inoculated in the same manner as described for VITEK-2 compact system, evaluation of each organism's growth pattern in the presence of the antimicrobial in relation to growth control well. Several parameters based on the growth characteristics observed are used to provide appropriate input for MIC calculations. The MIC result must be linked to an organism identification to determine a category interpretation. A category interpretation of MIC will be reported according to the interpretations defined by Global 2012.

3. Results

The identified *Y. regensburgei* revealed disparity in biochemicals tests of Vitek - 2 system as shown in table (1).

Table (1) The results of biochemical tests of Vitek -2 system.

Test	Result
Ala-phe-pro-arylamidase, Adonitol, L-pyrrolydonyl-arylamidase, L- arabitol, D-cellobiose, Glutamyl arylamidase PNA, Fermentation/ glucose, D- maltose, β- xylosidase, β- alanine – arylamidase PNA, L- proline- arylamidase, Palatinase, Lipase, D-sorbitol, Sucrose, D- Tagatose, 5-Ketoguconate, A-Glucosidase, Glycine- arylamidase, B-N-acetyl galactosaminidase, α-Galactosidase, Glycine- arylamidase, Lysine decarboxylase, L-Histidine assimalation, B-Glucoronidase, Glu-Gly-Arg-arylamidase, L-lactate assimilation, ELLman, L-maltate assimalation	-VE
H2S production, β-N-acetyl- glucosaminidase, D- glucose, Gamma-glutamyl – transferase, β-glucosidase, D- mannitol, D-mannose, Tyrosine- arylamidase, Urease, D-Trehalose, Citrate-Na, L-Lactate alkalinization, Succinate alkalinization, Phosphotase, Ornithin decarboxylase, Coumorate, O/129 resistance({camp. Vibrio)	+VE

As stated in table (2) the bacterium showed differences in its susceptibility patterns where the bacterium was sensitive to most screened antibiotics used in Vitek 2 system on contrary the bacterium exhibited resitance to Nitrofurantoin, Tetracycline, Trimethoprim / Sulfamethaxazole.

Table (2) antibiogram of identified *Y. regensburgei* to different antibiotics tested by Vitek 2 system.

Antibiotic(s)	**MIC**	**Interpretation**
Amikacin, Ampicillin	**<=2**	**S**
Amoxicillin/ clavulanic acid, Imipenem	**4**	**S**
Aztreonam,Cefepime, Ceftriaxone, Gentamicin	**<=1**	**S**
Cefazolin, Piperacillin/ Tazobactam	**<=4**	**S**
Ciprofloxacin	**<= 0.25**	**S**
ESBL	**-VE**	**-**
Ertapenem	**1**	**S**
Levofloxacin	**0.5**	**S**
Meropenem	**<= 0.25**	**S**
Nitrofurantoin	**256**	**R**
Tetracycline	**< =16**	**R**
Trimethoprim / Sulfamethaxazole	**< = 320**	**R**

ESBL, Extended Spectrum β-Lactamase ; MIC, Minimum Inhibitory Concentration

4. Discussion

The results of this study revealed that this bacterium was motile, oxidase , indole and methyl red were negative while catalase, voges proskauer, citrate utilization and urease were positive.

Identification and biochemical properties founded on monotonous biochemical testing. The organism was found to be a motile which was weakly positive for catalase, oxidase-negative and able to utilize citrate (positive after 48 h) and had a triple sugar iron reaction of K/A with gas (indole, H2S and urease not produced). The non-lactose-fermenting organism was probably identified as Salmonella enterica serovar Paratyphi A (S.

Paratyphi) (Sarika *et al*.,2013). Abbott and Janda (1994) published that both identified *Y regensburgei* strains were oxidase negative and catalase and nitrate positive. The strain from case 1 was esculin positive and anaerogenic in glucose and cellobiose broths, while the strain isolated from case 2 was esculin negative and aerogenic in both carbohydrates. Similar differences in regards to acetate utilization and glycerol and raffinose fermentation by *Y. regensburgei* strains were also noted in both of their surveys (Hickman-Brenner *et al*.,1985 ; Kosako *et al*.,1991). Abbott and Janda (1994) going on to stated that both identified strain of *Y. regensburgei* exhibited similarity in all biochemical tests where these strain were negative for indole , voges-proskauer, malonate, adonitol, dulcitol, glycerol , m-inositol,lactose ,a-ch3-glucoside raffinose, d-sorbitol, sucrose, mucate, lipase and Dnase while these strains were positive for citrate utilization, lysine decarboxylase , arginine dihydrolase , growth in KCN, glucose, L-arabinose, cellobiose, d. Mannitol, L-rhamnose, D-xylose,acetate. The identified bacterium was negative to Indole production , H2S production , Adonitol , Lactose, Malonate, Urease production , D-Sorbitol , Sucrose, Aesculin, Lipase, Arginine decarboxylase , Phenylalanine deaminase , D-Raffinose furthermore the bacterium was positive to L-Arabinose , D-Cellobiose , ONPG, D-Glucose , D-Maltose , D-Mannitol , Citrate, Glucose fermentation , Xylose, L-Rhamnose , Ornithine decarboxylase , Lysine decarboxylase (Sarika *et al*., 2013). The present study showed that the identified bacterium was positive for H2S production, β-N-acetyl- glucosaminidase, D- glucose, Gamma-glutamyl – transferase, β- glucosidase, D-mannitol, D-mannose, Tyrosine- arylamidase, Urease, D-Trehalose, Citrate-Na, L-Lactate alkalinization, Succinate alkalinization, Phosphotase, Ornithin decarboxylase, Coumorate, O/129 resistance (camp. Vibrio) while the babterium was negative to Ala-phe-pro-arylamidase, Adonitol, L-pyrrolydonyl-arylamidase, L-arabitol, D-cellobiose, Glutamyl arylamidase PNA, Fermentation/ glucose, D- maltose, β- xylosidase, β- alanine – arylamidase PNA, L- proline- arylamidase, Palatinase, Lipase, D-sorbitol, Sucrose, D- Tagatose, 5-Ketoguconate, A-Glucosidase, Glycine- arylamidase, B-N-acetyl galactosaminidase, α- Galactosidase, Glycine-arylamidase, Lysine decarboxylase, L-Histidine assimalation, B- Glucoronidase, Glu-Gly-Arg-arylamidase, L-lactate assimilation, ELLman, L-maltate assimalation as shown in table (1). Sarika *et al*. (2013) mentioned that the antibiotic resistance pattern of identified bacterium vary between resistance to Penicillin, Cefoxitin, Colistin and sensitivity to most antibiotics embodied by Ampicillin, Amoxicillin–clavulanate, Erythromycin, Ceftazidime, Ceftriaxone, Ciprofloxacin, Nalidixic acid, Gentamicin, Amikacin, Netilmycin, Piperacillin–tazobactam, Imipenem, Ertapenem, Meropenem, Tigecycline this was greatly agreed with the results of this study which revealed that the identified bacterium was resistant to Nitrofurantoin, Tetracycline, Trimethoprim / Sulfamethaxazole moreover this bacterium was sensitive to most screened antibiotics represented by Amikacin, Ampicillin, Amoxicillin/ clavulanic acid, Imipenem, Aztreonam,Cefepime, Ceftriaxone, Gentamicin, Cefazolin Piperacillin/ Tazobactam, Ciprofloxacin, Ertapenem, Levofloxacin, Meropenem whereas the bacterium was not produce extended spectrum beta lactamase. Kosako *et al.* (1984) mentioned that the epidemiological and clinical significance of this organism is not established. *Y. regensburgei* has been isolated from water, wells, salad and the intestinal tracts of insects and reptiles. It is rarely reported to cause human infections, with only sporadic case reports available in the literature. It has been isolated from leg wound, blood, upper respiratory tract, urine, faecal and synovial fluid samples (Stock *et al*., 2004; Abbott and Janda, 1994; Kosako *et al*., 1984; Hickman-Brenner *et al*., 1985; Yagu¨e Mun˜oz *et al*., 1989). Biochemically, *Y. regensburgei* closely resembles *Hafnia alvei* and *Salmonella enterica* (Stock *et al*., 2004); nevertheless, DNA relatedness values and other studies have showed that they are separate species (Kosako *et al*., 1984; Hickman-Brenner *et al*., 1985). The clinical importance of *Y. regensburgei* as a human pathogen is not well definite. This may be attributed to a lack of resources in routine clinical laboratories for its identification. Only five clinical cases of human infection have been reported worldwide. In 1993, an isolate was isolated from a left-knee wound. The isolate was initially forwarded to the local county public health laboratory and then to the Microbial Diseases Laboratory, where it was identified as *Y. regensburgei*. In 1994, gastrointestinal bleeding case pateint where blood culture was done and a gram-negative rod was identified. The strain was identified by the local laboratory as a possible Salmonella sp. Further laboratory diagnosis by the Microbial Diseases Laboratory using an alcohol-treated antigen shown no agglutination in Salmonella antiserum. Biochemical analysis identified this strain as *Y regensburgei* (Abbott and Janda, 1994), the bacterium were isolated from venous ulcer wound infection (Fajardo Olivares *et al.,* 2005), abdominal abscess and septic shock (Fill and Stephens, 2010) , soft tissue infection with bacteraemia (Lo *et al.,* 2011) and Sarika *et al*. (2013) present a case report of a bloodstream infection due to *Y. regensburgei* from India. An association between *Y. regensburgei* infection and some form of immune suppression has been observed in all these reports, such as alcohol intake, chronic renal failure, diabetes mellitus, adenocarcinoma and the use of steroids and immunosuppressive agents.In return for this current study revealed that the identified bacterium was isolated from chronic renal infection . Furthermore, all cases were reported in adults or elderly patients. Isolation of this species from water from wells, insect intestines, human upper respiratory tract, faeces and urine may suggest its ability to survive in these locations; however, the source and route of transmission is unclear (Kosako *et al.,* 1984; Hickman-Brenner *et al*., 1985).

Conclusion

Our conclusion that attention must drive towards the emerging of new agent of urinary tract infection represented by *Yokenella regensburgei* other than the common known agents which could indicate the shifting in pathogenicity of these agent .

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