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Isolation and Characterisation of a Novel *Nesterenkonia* species from a Human Bloodstream Infection

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Objectives

Nesterenkonia spp constitute a genus of bacteria within the *Micrococcaceae*. Eleven species:
N. haolobia, N. lacusekhonesis, N. halotorerans, N. xinjiangensis, N. lutea, N. sandarakina,
N. aethiopica, N. jeotgali, N. halophilia, N. flava and *N. alba* have been formally described, all recovered from environmental samples such as soil, salt lakes and industrial wastewater. In this study we describe a new member of the genus, designated strain NK1^T, isolated from the bloodstream of a human cancer sufferer.

Methods

NK1^T was isolated from a blood culture taken during an episode of febrile neutropenia from a patient with Hodgkins lymphoma. Gram-positive colonies recovered after aerobic incubation for 48 hrs on blood agar could not be identified using commercial API staph kits (Biomerieux) or a MALDI-Tof mass spectrometry system (Bruker) (Figure 1). Partial 16S rDNA sequencing (nucleotides 8 – 1541) and a genus specific PCR were used to identify the organism as a member of the genus *Nesterenkonia*. Taxonomic and phenotypic studies were undertaken to compare the properties of NK1^T to those peviously reported for existing *Nesterenkonia* spp (1). The 16S rDNA sequence was cloned in *E. coli* (pCR2.1 TOPO vector) sequenced and a phylogenetic tree constructed (Figure 2). Growth characteristics, pH and salt tolerance and the ability to utilise, ferment and degrade organic and inorganic compounds were determined using standard microbiological tests on colonies grown overnight on Luria-Burtani media. Susceptibility testing was performed by a disc diffusion method on Isosensitest agar and interpreted according to EUCAST zone diameter breakpoints for *Staphylococcus* spp.

Results

The 16S rDNA sequence of NK1^T was found to be unique but most closely related (98 %) to that of *N. lacusekhonesis*. NK1^T appeared as short Gram-positive rods and formed **white colonies** after growth on blood agar for 48 hrs (Figure 1B). The optimal temperature for **growth** was **30°C** and the organism was able to grow in **5 % salt** and at **pH 6.5 – 12. Biochemical reactions** distinguishing the organism from other *Nesterenkonia* spp included **lack of motility**, a **negative oxidase test**, **production of urease**, **utilisation of citrate**, **arabinose**, **cellobiose**, **mannose** and **sucrose** as carbon sources and **fermentation** of **xylose** and **mannitol** (Table 1). The isolate was susceptible to penicillins, tetracyclines, aminoglycosides, quinolones and glcyopeptides.

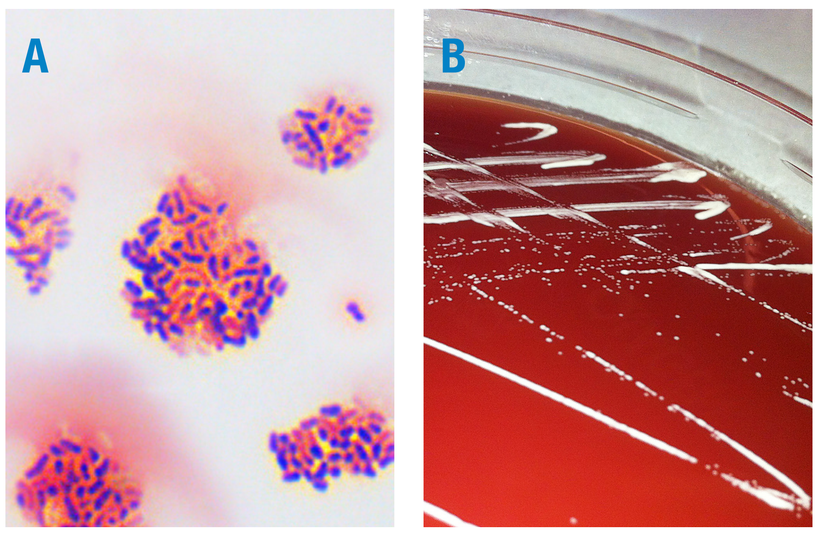
Table 1

Taxa: 1, strain NK1^T (*Nesterenkonia pheeae sp. nov.*); 2, *N. flava*; 3, *N. aethiopica*; 4, *N. xinjiangensis*; 5, *N. lacusekhoensis*; 6, *N. halobia*; 7, *N. lutea*; 8, *N. halotolerans*; 9, *N. sandarakina*; 10, *N. jeotgali*. Data for reference strains were taken from Luo et al (2008). +, Positive reaction; w, weak reaction; -, no reaction; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10
Morphology	**Short rods**	Short rods	Short rods	Short rods	Short rods	Cocci	Cocci	Cocci	Cocci	Cocci
Pigmentation	**White**	Yellow	Yellow	Light yellow	Bright yellow	Colourless	Light yellow to primrose yellow	Orange-yellow	Orange-yellow	Light yellow
Motility	**-**	-	-	-	-	-	+	+	-	-
Optimal Temperature (°C)	**30**	40-42	30-37	28	27-33.5	30	28	28	28	25-30
pH tolerence	**6.5-12**	8.0-12.0	7.0-11.0	7.0-12.0	7.5-9.5	<6.0-10.0	6.5-10.0	7.0-9.0	5.0-12.0	6.0-8.5
NaCl tolerance (%)	**0-5**	0-10	3-Dec	0-25	0-15	May-23	0-20	0-25	Jan-16	0-16
Catalase activity	**+**	+	+	+	+	+	+	+	+	ND
Oxidase activity	**-**	-	+	-	-	+	-	-	-	-
Urease	**+**	ND	ND	ND	ND	ND	ND	ND	ND	ND
H2S prodcution	**-**	ND	ND	ND	ND	ND	ND	ND	ND	ND
Indole production	**-**	ND	ND	ND	ND	ND	ND	ND	ND	ND
ONPG test	**-**	ND	ND	ND	ND	ND	ND	ND	ND	ND
Nitrate reduction	**-**	-	ND	-	-	-	+	-	-	-
Citrate test	**+**	-	-	ND	w	-	ND	ND	ND	ND
Voges-Proskauer reaction	**-**	-	ND	ND	-	+	-	+	-	+
Hydrolysis of :										
Gelatin	**-**	+	+	+	-	-	-	+	+	-
Tween 80	**ND**	+	ND	ND	-	-	-	-	-	-
Utilization of:										
D-Glucose	**-**	+	ND	+	+	ND	w	+	+	+
D-Xylose	**-**	-	-	+	ND	+	+	-	+	+
L-Arabinose	**+**	+	+	+	ND	+	w	-	w	+
Cellobiose	**+**	+	ND	+	ND	ND	+	-	+	+
D-Fructose	**-**	+	ND	+	+	ND	+	+	+	+
D-Mannose	**+**	+	-	+	+	-	+	+	+	w
Sucrose	**+**	+	w	+	+	-	+	+	+	+
Maltose	**-**	+	ND	+	+	ND	+	+	+	+
Acid production from										
D-Galactose	**-**	-	-	-	-	w	+	-	+	+
Lactose	**-**	-	-	-	-	+	+	-	-	-
Trehalose	**w**	-	-	-	+	-	+	-	-	w
D-Xylose	**+**	-	-	-	-	+	+	-	+	+
Mannitol	**+**	-	-	-	-	+	+	-	+	+

Figure 1

A Gram stain appearance of NK1^T
B Colonial morphology on blood agar
C MALDI-Tof mass spectrometry trace



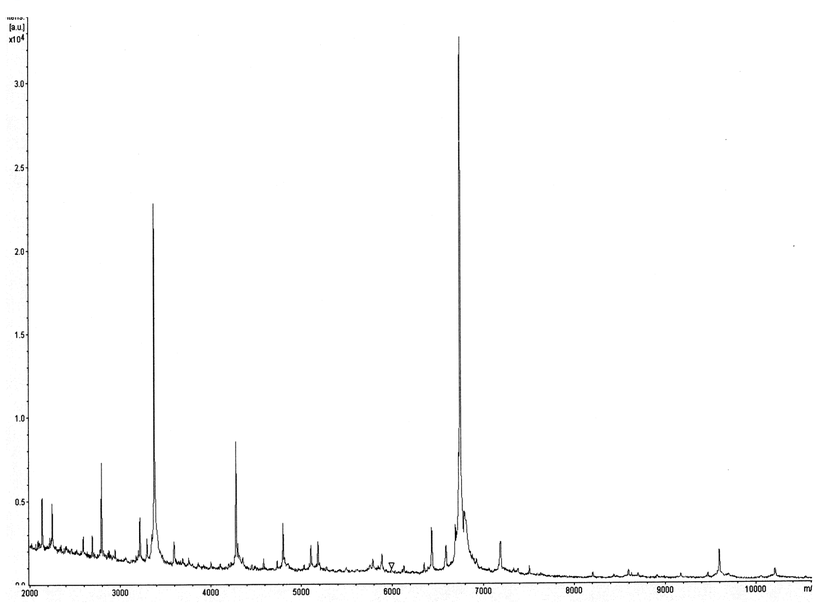
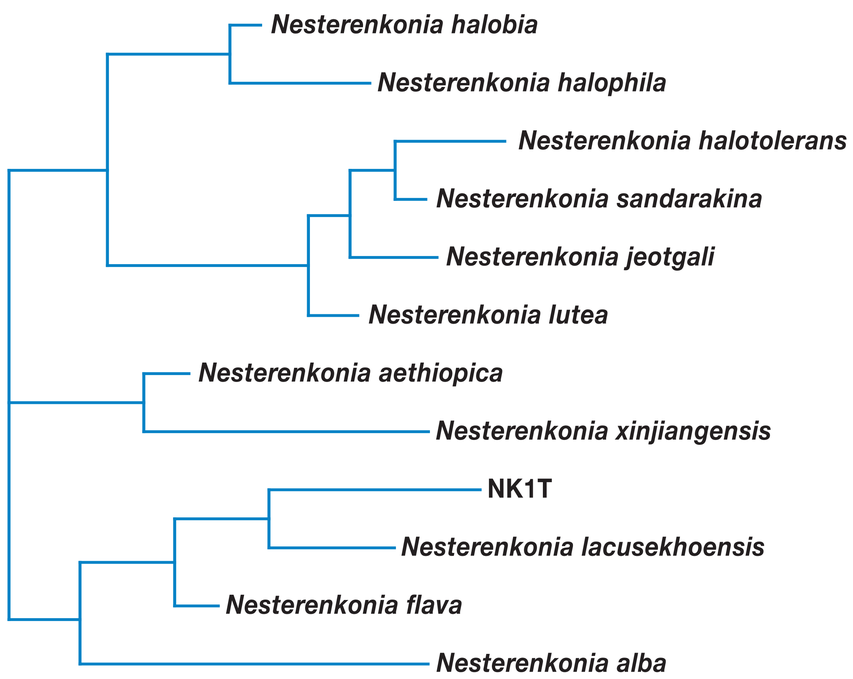


Figure 2

Neighbour joining Phylogenetic denodrogram of 16s rRNA sequences of 10 *Nesterenknonia* species



Conclusion

We describe a novel bacterial species belonging to the genus *Nesterenkonia*, (NK1^T) provisionally named *Nesterenkonia pheeae* sp. Nov. This is the 1st report of a human bloodstream infection due to *Nesterenkonia* which could be an emerging pathogen in immunocompromised patients.

References

1 Luo HY, Miao LH, Fang C, Yang PL, Wang YR, Shi PJ, Yao B, Fan YL. Nesterenkonia flava sp. nov., isolated from paper-mill effluent. *Int J Syst Evol Microbiol*. 2008 Aug;58(Pt 8):1927-30.