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# Pulmonary Nocardiosis; Similarity to Tuberculosis (A Bacteriological and Proteomics Study)

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## ABSTRACT

**Objective**: The aims of the present study were to decide the occurrence of nocardia spp. among Sudanese patients suspected with tuberculosis and to investigate all proteins expressed by the genome of *Nocardia africana* (formerly isolated from patients with pulmonary infection misdiagnosed as MDR and their structures and functions compared to Mycobacterium tuberculosis.

**Materials and Methods**: Three hundred and twenty-nine patients, presented with pulmonary infection were included in this study. Those patients were examined for the presence of acid- fast bacilli. Two tubes of Lownstein- Jensen (L.J) medium were inoculated with 20 µl of the neutralized sputum sample. All cultures were incubated at 37°C for 8 weeks before being discarded. Phenotypic characterizations were performed. For nocardia proteom poly acrylamide gele lectrophoresis (PAGE)-based analyses of the four nocardia strains *N. farcinica* SD1828, *N. africana* SD 925, and N. asteroides N317 are discussed. In-gel tryptic digestion of these isolates was also performed, then the resulting peptides were introduced to MALDI-TOF peptide mass fingerprints were searched using MASCOT software.

**Results:** Ten isolates showed rapid growth pattern within 2-3 days after inoculation, further conventional methods suggested that all these isolates were belonging to the family nocardiacea. Two Dimentional Poly Acrylamide Gel Electrophoresis (2D-PAGE) using pH strips 3-10 revealed that the soluble proteins were visible in a much smaller pI range. All strains exhibited similar protein distributions. A similarity analysis revealed that mycobacterium sequences are of high relevance for the investigated strains.

**Conclusions:** Nocardia revealed considerable occurrence among patients with pulmonary infections (3.3%) giving clinical symptoms similar to those occur by M. tuberculosis infection, this may be due to similarities in functional proteins expressed by their genomes. This finding suggested that pulmonary nocardiosis might occur in patients who suffer from chronic lung disease in Sudan. It is important, therefore, that clinicians in Chest Units should consider this condition, especially when patients with respiratory infections fail to respond to antitubercular therapy.

Nocardiae are Gram-positive aerobic actinomycetes, which are predominantly saprophytic (Orchard, 1981) but also include species forming parasitic association with animals and plants (Goodfellow, 1992). They are in the same family as clinically and industrially important genera such as Mycobacterium, Streptomyces,

Corynebacterium and Rhodococcus and they are known to cause a variety of infections in humans and animals. Nocardiae cause a variety of suppurative infections of humans and animals (Ishikawa, 2004; Mogahid *et al*., 2007). Human infections may be distinguished clinically into cutaneous, subcutaneous, and lymphocutaneous nocardiosis; extrapulmonary nocardiosis; pulmonary nocardiosis; and systemic nocardiosis involving two or more body sites. The incidence of such infections is not known, although nocardiosis has been reported in most regions of the world. Nocardial infections of the internal organs in nontropical countries are mainly caused by *Nocardia asteroides, N. farcinica*, and *N. nova*; relatively few are caused by *N. brasiliensis, N. otitidiscaviarum, N. pseudobrasiliensis*, and *N. transvalensis*. There have been isolated reports of pulmonary nocardiosis from tropical countries caused by *N. asteroides*, *N. brasiliensis, N. farcinica, N. otitidiscaviarum,* and *N. transvalensis*, (Koltzscher *et al*., 2003; Bauer *et al*., 1966; Gang *et al*., 2005). According to information from the Center of Disease Control and prevention 80% of cases present as invasive pulmonary infection, disseminated disease, or brain abscess, and 20% present as cellulitis. *Nocardia asteroides* causes at least 50% of invasive infections. In the United States, estimated 500-1,000 new cases of nocardia infection occur annually. The number of cases has increased with the overall rise in the number of severely immunocompromised persons. Diagnosis is a major challenge. *N. farcinica* frequently is resistant to antimicrobial agents, including the drug of choice trimethoprim-sulfamethoxazole, and has been demonstrated to be more virulent in an animal model. A new combination drug therapy (sulfonamide, ceftriaxone, and amikacin) has shown promise for infections difficult to treat. The hope is that the application of newer molecular diagnostic and subtyping methods may assist in earlier diagnosis and outbreak investigation (www.cd.gov).

Recently, proteomics have been introduced as a new field of research for ultra study of protein structure and function Proteomics is the study of all proteins expressed by a genome. It involves the identification of proteins in the body and the determination of their role in physiological and pathophysiological functions. By proteomics, many new proteins have been identified in different microorganisms and their functions have been well understood (Cutler *et al*,. 1999, Gorg *et al*., 2000).

Two-dimensional (2-D) gel electrophoresis of pulse-labeled proteins revealed a global analysis of protein synthesis and turnover in *Escherichia coli* (Weichart *et al*., 2003). Comparative proteomics of the human pathogen *Campylobacter jejuni* revealed an important first step in characterizing strain differences potentially responsible for different disease outcomes associated with this organism (Brubacher *et al*., 2003). Several studies subjected *Mycobacterium tuberculosis* to comprehensive proteomic analysis. Culture supernatant proteins of virulent *Mycobacterium tuberculosis* $H_{37}$Rv and attenuated *Mycobacterium bovis* BCG were comprehensively analyzed using proteomics techniques (Mattow *et al*., 2001). In another study, proteomics revealed open reading frames in Mycobacterium tuberculosis $H_{37}$Rv not predicted by genomics (Peter *et al*., 2003). This clearly showed that, analysis of proteins directly may come up with new knowledge which may completely alter the approaches to disease diagnosis, control and treatment. Definitive diagnosis of nocardiosis depends on the isolation and identification of the causative organism from clinical materials. These procedures are not straightforward; hence the true incidence of the disease is masked, a problem which is compounded by poor documentation. Generally, nocardia cases are difficult to diagnose (Garcia-Benitez *et al*., 2001).

## MATERIALS AND METHODS

### Collection of the Samples

Three hundred and twenty- nine patients, who were attending Abu-Anga Teaching Hospital, El-Shaab Teaching Hospital and the National Health Laboratory, in the Sudan during the period from October 2004 to January 2006, were examined for the presence of acid-fast bacilli. They were suspected of having tuberculosis infection according to the symptoms. Most of the patients had either not responded to treatment with antitubercular drugs or had responded and then relapsed. Sputum samples were collected according to WHO criteria in sterile, plastic wide-mouthed, strong leak-proof containers. Following treatment with the digestion-decontamination procedure of Roberts *et al*., 1991, the sputum samples were concentrated by centrifugation and the resultant preparations were used to inoculate Lowenstein-Jensen (LJ) slopes, which were incubated at 37°C for 14 days and then used to make smears, which were examined with a standard Ziehl-Neelsen acid-fast stain.

### Phenotypic Characterization

Ten of the LJ slopes supported the growth of small orange filamentous colonies, which were considered to be typical of nocardiae. The isolates, which were designated SD1001, SD1002, SD1002, SD1003, SD1004, SD1005, SD1006, SD1007, SD1008, SD1009 and SD1010, were subcultured and maintained on glucose-yeast extract agar (GYEA) slopes at room temprature. The ten isolates were examined for a range of phenotypic properties described by Isik *et al*., 1999. Standard procedures were also used for the extraction and analysis of mycolic acids as described by Minnikin, *et al*., 1975 and *Nocardia africana* strains used as controls.

### Preparation on Nocardia Lysate

The test strains were cultivated on GYEA medium (Appendex I1) for 2-3 days at 37°C then checked for purity. Nocardia species were then harvested, inactivated in the water path at 90oC for one hour and the cells were washed with sterile phosphate buffer saline (P.B.S) and then centrifuged at 15000 rpm for 5 minutes. Supernatants were discarded and the pellet was washed by resuspending in MilliQ water and centrifuged again at 10000 rpm for 10 minutes, and the supernatant was discarded.

Then, 1ml of urea lysis buffer 8 M (Appendix IV) was added to each sample in eppendorf tubes in addition to 100 µl of protease inhibitor (CALBIOCHEM, Cat No 539134), 20 µl of (500 U) benzonase (Novagen Lot N 62211-1) and 0.5gm of silica beads (0.1mm, Cat No 11079101 Z- Biospec products, Inc). The mixture was then vortexed for one minute (Vortex Genie 2 Model G.560E Bohemia USA).

Samples were then lysed by freezing in liquid nitrogen and thawing in 37oC in waterpath, this step was repeated for 5 times. Samples were then put on a shaker (Type MM 300 cat No 85720 GmbH and co KG, Germany) with a frequency of 3000 per minute for 30 minutes. Step of freeze-thawing was repeated again for other 5 times and the shaking step was also repeated as previously described. Samples were then spin down at 15000 rpm for 5 minutes, supernatant (which contain the soluble proteins) was collected and protein concentration was measured with spectrophotometer (Model 8024-0600, From Pharmacia Biotech, England).

### Two Dimensional Gel Electrophoresis

Seventy (70) µg of protein were Loaded on immobilized pH gradients (I PG) dry Strip (pH 4-7, 7 cm) together with IPG buffer (pH 3- 10) and 20 mM DTT), overnight passive rehydration was performed for the dry strip then Ettan I PG phor (Amersham Bioscience) was used to a achieve First dimension Iso Electrical Focusing. A second dimension SDS – PAGE

was done by using Hofer miniv E8 ×9cm gels device accrding to Laemmli.1970

**Tryptic Digestion and Mass Spectrometric Sequencing**

In-solution tryptic digestion of nocardia isolates lysate was also performed; a total of 4µl (containing approximately 250 µg) of the lysate was dry with vacuum centrifuge, the dry protein was redisolved in 250 µl of 8 M urea, 0.4 M $N_4HCO_3$ followed by 25µl of 45 mM dithiothereitol the mixture was incubated at 50 $^0$ C fore 15 minutes, 25 µL of 100 mM Iodoactamide were added and the mixture was incubated at ambient temperature in darkness for additional 15 minutes, 700 µL of deionized water was added together with 5% (w/w) trypsin (12.5 µg).The digestion was performed at 37$^0$ C overnight in darkness. Resulting peptides were then desalted by using Zip Tip column (microbed $C_{18}$; Millipore, USA), then the peptides were introduced to mass spectrophotometer (QSTAR from Applied Biosystem), peptide mass fingerprints were searched using MASCOT software.

## RESULTS

**Bacteriology Results**

All positive smears showed positive growth on LJ medium after 2-21 days post inoculation. The colonial morphology of 319 (97%) appeared as rough, friable, warty, granular and grey in color with irregular margins and showed the appearance of AFB when stained again (indirect smear) with ZN staining procedure for more confirmation. The 319 isolates were initially identified as members of the *Mycobacterium tuberculosis* complex.

Ten (3%) of the LJ slopes reveled the growth of small orange filamentous colonies, which were tentatively considered to be nocardiae (Fig. 1).

The 10 nocardia isolates, which were then designated SD1001, SD1002, SD1003, SD1004, SD1005, SD1006, SD1007, SD1008, SD1009 and SD10010 were subcultured and maintained on glucose-yeast extract agar (GYEA) slopes at room temperature and as suspensions of mycelial fragments in glycerol (20% [vol/ vol]) at −70°C. The 10 isolates were studied phenotypically.

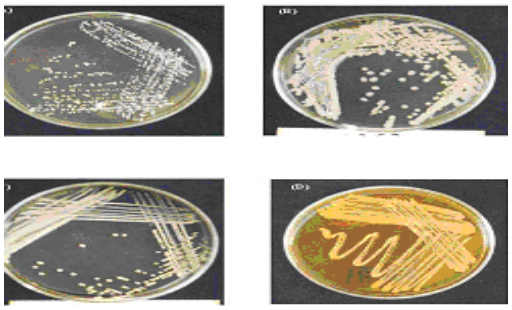

Fig. 1: Growth of *N. africana* SD 1002 (A); SD 1003 (B); SD 925 (*C*); *N. farcinica* SD 1819 (D); on glucose yeast extract agar medium.7 days old cultures.

**Biochemical tests for nocardia isolates**

Selected biochemical tests were performed. The result for these tests showed that all the strains utilize glucose by oxidation pathway and that they were all catalase positive and also positive for urea. Concerning growth at 45°C, 7 out of the ten were positive (70%). Only 2 (20%) were positive for manitol and rhaminose as well as starch whilst all the isolates were negative for xanthine, casein, tyrosine, sorbitol, arabinose and citrate. Regarding mycolic acids, all tested isolates showed the standard patterns of mycolic acid components using thin layer chromatographic technique.The tested strains were found to have phenotypic properties typical of members of the genus nocardia ( Figs 1 and 2).

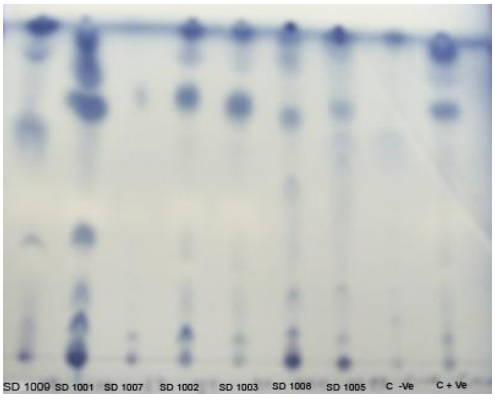


Fig. 2: Thin layer chromatography (TLC) (10x10cm), run twice in a solvent containing Toluen–Aceton (97:3, V/V), C +Ve*: N. africana* SD 925, C –Ve: *S. aurues*.

**Proteomic Results**

The results of 2-D gel electrophoresis revealed a total of hundreds of protein spots for nocardia lysate. The molecular weights range between 25 -75 kDa and the Iso-electric point (pI) of these range between 4–7 (Figs 3 and 4).

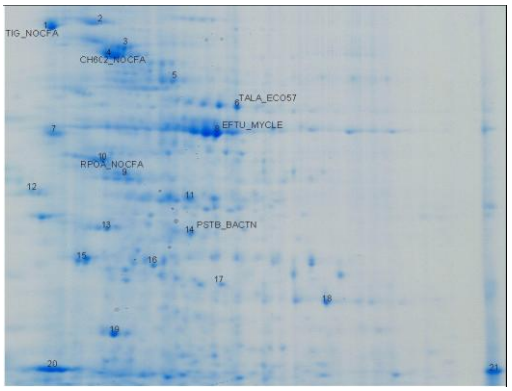


Fig. 3: Representative spot assignments for *N. farcinica* SD1828, pH 4-7, MW 25-75 kDa.

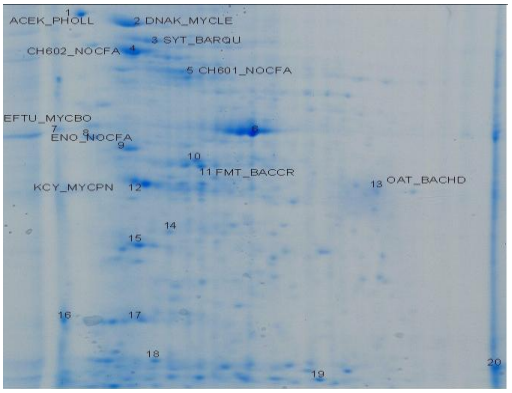


Fig. 4: Representative spot assignments for *N. africana*. SD 925, pH 4-7, MW 25-75 kDa.

On the other hand, different peptides were detected by mass spectrometer. Alignment of those peptides was performed on Swiss-Prot. The resulting data revealed highly significant homology with *Mycobacterium tuberculosis* (set of Table 1).

## CONCULOSION

A relatively large sample size (329) was analyzed in this study compared with previous studies in Sudan conducted in the same field and nocardia species has constituted 3% of all isolate. A significant resistant pattern (40%) were observed through these ten (10) isolates, hence more attention should be drawn towards patients who did not respond to anti-tuberculosis therapy, as other pathogens, including *Nocardia* spp. may be the cause of the infection.

Hundreds of nocardia protein spots were captured on stained gel. Some proteins were identified in a gel digest using Q-STAR instrument analysis of the results showed significant similarities between nocardia and *Mycobacterium tuberculosis*; this may provide evidences for best understanding of the pathogenesis and increase the knowledge with respect to disease treatment and vaccination.

Table 1/1: Elongation factor Tu of Mycobacterium leprae EFTU_MYCLE (P30768)

| Description | SwissProt Accession # | Organism |
|---|---|---|
| _MYCPA | Q73SD1 | *Mycobacterium paratuberculosis* |
| EFTU_MYCTU | P0A558 | *Mycobacterium tuberculosis* |
| EFTU_MYCBO | P0A559 | *Mycobacterium bovis* |
| _MYCSS | Q1BDD3 | *Mycobacterium sp. (strain MCS)* |
| _9MYCO | Q1T9Z3 | *Mycobacterium sp. KMS* |
| _9MYCO | Q1TV69 | *Mycobacterium sp. JLS* |
| _MYCVN | Q262Y2 | *Mycobacterium vanbaalenii PYR-1* |
| _MYCFV | Q27DQ3 | *Mycobacterium flavescence* |
| _NOCFA | Q5YPG4 | *Nocardia farcinica* |

Sequence comparison EFTU_MYCLE and Q5YPG4_NOCFA

Query: 1 MAKAKFERTKPHVNIGTIGHVDHGKTTLTAAITKVLHDKFPNLNESRAFDQIDNAPEERQ 60
+AKAKFERTKPHVNIGTIGHVDHGKTTLTAAITKVL DK+P+LN+S AFDQID APEE+
Sbjct: 20 VAKAKFERTKPHVNIGTIGHVDHGKTTLTAAITKVLADKYPDLNQSFAFDQIDKAPEEKA 79

Query: 61 RGITINISHVEYQTEKRHYAHVDAPGHADYIKNMITGAAQMDGAILVVAATDGPMPQTRE 120
RGITINISHVEYQTEKRHYAHVDAPGHADYIKNMITGAAQMDGAILVVAATDGPMPQTRE
Sbjct: 80 RGITINISHVEYQTEKRHYAHVDAPGHADYIKNMITGAAQMDGAILVVAATDGPMPQTRE 139

Query: 121 HVLLARQVGVPYILVALNKSXXXXXXXXXXXXXXXXXXXXXQEFDEDAPVVRVSALKAL 180
HVLLARQVGVPYILVALNK+ QEFDE+APVVRVS LKAL
Sbjct: 140 HVLLARQVGVPYILVALNKADMVDDEEILELVEMEVRELLAAQEFDEEAPVVRVSGLKAL 199

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EGD KWV+SV LMDAVDESIP PVRETDKPFLMP+EDVF
Sbjct: 200 EGDPKWVKSVEDLMDAVDESIPDPVRETDKPFLMPIEDVFTITGRGTVVTGRVERGIINV 259

Query: 241 XXXXXXXXXXQXXXXXXXXGVEMFRKLLDQGQAGDNVGLLLRGIKREDVERGQVVIKPGT 300
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Query: 301 TTPHTEFEGQVYILSKDEGGRHTPFFNNYRPQFYFRTTDVTGVVTLPEGTEMVMPGDNTN 360
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Sbjct: 320 TTPHTEFEGQAYILSKDEGGRHTPFFNNYRPQFYFRTTDVTGVVTLPEGTEMVMPGDNTE 379

Query: 361 ISVTLIQPVAMDEGLRFAIREGGRTVGAGRVVKIIK 396
+SV LIQPVAM+EGLRFAIREGGRTVGAGRV KIIK
Sbjct: 380 MSVKLIQPVAMEEGLRFAIREGGRTVGAGRVTKIIK 41

Table 1/2: Chaperone protein dnaK, HSP70 of *Mycobacterium paratuberculosis* DNAK_MYCPA (Q00488)

| Description | SwissProt Accession # | Organism |
|---|---|---|
| DNAK_MYCLE | P19993 | *Mycobacterium leprae* |
| DNAK_MYCTU | P0A5B9 | *Mycobacterium tuberculosis* |
| DNAK_MYCBO | P0A5C0 | *Mycobacterium bovis* |
| _MYCVN | Q25TL7 | *Mycobacterium vanbaalenii PYR-1* |
| _MYCFV | Q27CE8 | *Mycobacterium flavescence PYR-GCK* |
| _MYCSS | Q1BEV1 | *Mycobacterium sp. strain MCS* |
| _9MYCO | Q1TWF2 | *Mycobacterium sp. JLS* |
| _9MYCO | Q1TDC6 | *Mycobacterium sp. KMS* |
| DNAK_NOCFA | Q5YNI0 | *Nocardia farcinica* |

Sequence comparison DNAL_MYCPA and DNAK_NOCFA

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ARAVGIDLGTTNSVVAVLEGG+PVVVANSEGSRTTPSIVAFA+NGEVLVGQPAKNQAVTN
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Query: 181 EIGEGVVEVRATSGDNQLGGDDWDDRIVNWLVDKFKGTSGIDLTKDKMAMQRLREAAEKA 240
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Sbjct: 537 SKVEAAIAEANEALAGTDIAAVKAAVEKLATESQALGQAIYEAQGAD 583

**Table 1/3:** 60 kDa chaperonin 1 of *N. farcinica* CH601_NOCFA (Q5Z1F9)

| Description | SwissProt Accession # | Organism |
|---|---|---|
| _RHOSR | Q0S3B9 | *Rhodococcus sp. strain RHA1* |
| _MYCSS | Q1BCW7 | *Mycobacterium sp. strain MCS* |
| _9MYCO | Q1TR57 | *Mycobacterium sp. JLS* |
| _9MYCO | Q1TCR3 | *Mycobacterium sp. KMS* |
| _MYCVN | Q263S8 | *Mycobacterium vanbaalenii PYR-1* |
| _MYCFV | Q27E56 | *Mycobacterium flavescence PYR-GCK* |
| CH601_MYCPA | P60545 | *Mycobacterium paratuberculosis* |
| CH601_MYCTU | P0A518 | *Mycobacterium tuberculosis* |
| CH601_MYCBO | P0A519 | *Mycobacterium bovis* |
| CH601_MYCLE | P37578 | *Mycobacterium leprae* |

Sequence comparison CH601_NOCFA and Q0S3B9_RHOSR and Q1BCW7_MYCSS

Query: 1 MAKQIEFDEKARRALERGVDKLADAVKVTLGPRGRHVVLAKAFGGPTVTNDGVTIARDID 60
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Query: 361 EKLEERLXXXXXXXXXXXXXXXTETALKERKYRVEDAVSAAKAAVDEGIVPGGGTALVQA 420
EKLEERLTET LKERK+RVEDAV+AAKAAV EGIVPGGG+ALVQA
Sbjct: 361 EKLEERLAKLAGGVAVIKVGAATETDLKERKFRVEDAVNAAKAAVAEGIVPGGGSALVQA 420
EKLEERLTET LK+RK VEDAVSAAKAAV+EGIV GGG ALVQA
Sbjct: 361 EKLEERLAKLAGGVAVIKVGAATETDLKKRKEAVEDAVSAAKAAVEEGIVTGGGAALVQA 420

Query: 421 ATKLVELRDSLSGDEAVGVEVVRKALEAPLFWIASNAGLDGAVVVSKVAEGKE--GFNAA 478
+T+L+L+GDEA GV+VVR+AL+APL+WIASNAGLDG+VV SKVAE +GFNAA
Sbjct: 421 STELAD-NLGLTGDEATGVKVVREALQAPLYWIASNAGLDGSVVTSKVAEQPKGHGFNAA 479
L LR S+SGDEA+GVEVAL APL+WIA+NAGLDG+VVV+KV+E +GFNAA
Sbjct: 421 RKALDSLRGSVSGDEALGVEVFNSALSAPLYWIATNAGLDGSVVVNKVSELPAGQGFNAA 480

Query: 479 TLSYGDLLTDGVVDPVKXXXXXXXXXXXXXXMVLTTESAVVDKPAEEAEDHSHHGHAH 536
TL+YGDLL DGVVDPVKM+LTTESAVV+KPAEE E + HGH+H
Sbjct: 480 TLTYGDLLADGVVDPVKVTRSAVVNAASVARMILTTESAVVEKPAEENEQQTGHGHSH 537
TL +GDLL DGVVDPVKMVLTTE+A+VDKPAEE EDH HHGHA
Sbjct: 481 TLEFGDLLADGVVDPVKVTRSAVLNAASVARMVLTTETAIVDKPAEE-EDHGHGHHHGHA 539

Query: 536 H 536
Sbjct: 540 540
H
Sbjct: 540 H 540

Table 1/4: 60 kDa chaperonin 2 of *N. farcinica* CH602_NOCFA (Q9AFA6)

| Description | SwissProt Accession # | Organism |
|---|---|---|
| CH60_COREQ | Q93QI2 | *Corynebacterium equii (Rhodococcus equi)* |
| _RHOSR | Q0SET3 | *Rhodococus sp. strain RHA1* |
| _MYCFV | Q27CR6 | *Mycobacterium flavescence PYR-GCK* |
| _MYCSS | Q1BEF6 | *Mycobacterium sp. strain MCS* |
| _9MYCO | Q1TWU6 | *Mycobacterium sp. JLS* |
| _MYCVN | Q267S7 | *Mycobacterium vanbaalenii PYR-1* |
| _9MYCO | Q1TDR8 | *Mycobacterium sp. KMS* |
| _9MYCO | Q8GAR8 | *Mycobacterium sp. 185-409* |
| CH60_NOCAS | Q9AFC5 | *Nocardia asteroides* |
| _MYCMR | Q8G8X0 | *Mycobacterium marinum* |
| CH602_MYCPA | P42384 | *Mycobacterium paratuberculosis* |

Sequence comparison CH602_NOCFA and CH60_NOCAS and CH602_MYCPA

```
Query: 1MAKTIAYDEEARRGLERGLNSLADAVKVTLGPKGRNVVLEKKWGAPTITNDGVSIAKEIE 60
       MAKTIAYDEEARRGLERGLNSLADAVKVTLGPKGRNVVLEKKWGAPTITNDGVSIAKEIE
Sbjct: 1MAKTIAYDEEARRGLERGLNSLADAVKVTLGPKGRNVVLEKKWGAPTITNDGVSIAKEIE 60
Query: 2    AKTIAYDEEARRGLERGLNSLADAVKVTLGPKGRNVVLEKKWGAPTITNDGVSIAKEIEL 61
            AKTIAYDEEARRGLERGLN+LADAVKVTLGPKGRNVVLEKKWGAPTITNDGVSIAKEIEL
Sbjct: 1    AKTIAYDEEARRGLERGLNALADAVKVTLGPKGRNVVLEKKWGAPTITNDGVSIAKEIEL 60

Query: 61  LEDPYEKIGAELVKEVAKKXXXXXXXXXXXXXXXXXXXXXXXREGLRNVAAGANPLGLKRGIE 120
           LEDPYEKIGAELVKEVAKK REGLRNVAAGANPLG KRGIE
Sbjct: 61  LEDPYEKIGAELVKEVAKKTDDVAGDGTTTATVLAQALVREGLRNVAAGANPLGRKRGIE 120
Query: 62   EDPYEKIGAELVKEVAKKXXXXXXXXXXXXXXXXXXXXXXXREGLRNVAAGANPLGLKRGIEK 121
            EDPYEKIGAELVKEVAKKREGLRNVAAGANPLGLKRGIEK
Sbjct: 61   EDPYEKIGAELVKEVAKKTDDVAGDGTTTATVLAQALVREGLRNVAAGANPLGLKRGIEK 120

Query: 121 KAVEAVTAKLLDTAKEVETKEQIAATAGISAGDASIGELIAEAMDKVGKEGVITVEESNT 180
           KAVEAVTAKLLDTAKEVETKEQIAATAGISAGDA+IGELIAEAMDKVGKEGVITVEESNT
Sbjct: 121 KAVEAVTAKLLDTAKEVETKEQIAATAGISAGDAAIGELIAEAMDKVGKEGVITVEESNT 180
Query: 122  AVEAVTAKLLDTAKEVETKEQIAATAGISAGDASIGELIAEAMDKVGKEGVITVEESNTF 181
            AVE VT  LL +AKEVETK+QIAATA ISAGD SIG+LIAEAMDKVG EGVITVEESNTF
Sbjct: 121  AVEKVTETLLKSAKEVETKDQIAATAAISAGDQSIGDLIAEAMDKVGNEGVITVEESNTF 180

Query: 181 FGLQLELTEGMRFDKGYISGYFVTDPERQEAVLEDPYILLVGSKVSTVKDLLPLLEKVIQ 240
           FGLQLELTEGMRFDKGYISGYF TDPERQEAVLEDPYILLVGSKVSTVKDLLPLLEKVIQ
Sbjct: 181 FGLQLELTEGMRFDKGYISGYFATDPERQEAVLEDPYILLVGSKVSTVKDLLPLLEKVIQ 240
Query: 182  GLQLELTEGMRFDKGYISGYFVTDPERQEAVLEDPYILLVGSKVSTVKDLLPLLEKVIQA 241
            GLQLELTEGMRFDKGYISGYFVTD ERQEAVLEDP+ILLV SKVSTVKDLLPLLEKVIQA
Sbjct: 181  GLQLELTEGMRFDKGYISGYFVTDAERQEAVLEDPFILLVSSKVSTVKDLLPLLEKVIQA 240

Query: 241 AGKPLLIIAEDVEGEALSTLVVNKIRGTFKSVAVKAPGFGDRRKAQLADIAILTGGEVIS 300
           AGKPLLIIAEDVEGEALSTLVV KI GTFKSVAVKAPG GDRRKAQLADIAILTGG+VIS
Sbjct: 241 AGKPLLIIAEDVEGEALSTLVVKKILGTFKSVAVKAPGGGDRRKAQLADIAILTGGQVIS 300
Query: 242  GKPLLIIAEDVEGEALSTLVVNKIRGTFKSVAVKAPGFGDRRKAQLADIAILTGGEVISE 301
            GKPLLIIAEDVEGEALSTLVVNKIRGTFKSVAVKAPGFGDRRKA L D+AILTGG+VISE
Sbjct: 241  GKPLLIIAEDVEGEALSTLVVNKIRGTFKSVAVKAPGFGDRRKAMLQDMAILTGGQVISE 300

Query: 301 EEVGLSLETAGIELLGQARKVVVTKDETTIVEGAGDAEAIKGRVAQIRTEIENSDSDYDR 360
           EEVGLSLETAGIELLGQARKVVVTKDETTIVEGAGDAEAI GRV+QIR EIENSDSDYDR
Sbjct: 301 EEVGLSLETAGIELLGQARKVVVTKDETTIVEGAGDAEAIAGRVSQIRAEIENSDSDYDR 360
Query: 302  EVGLSLETAGIELLGQARKVVVTKDETTIVEGAGDAEAIKGRVAQIRTEIENSDSDYDRE 361
            EVGLSLE+A I LLG+ARKVVVTKDETTIVEGAGD++AI GRVAQIRTEIENSDSDYDRE
Sbjct: 301  EVGLSLESADISLLGKARKVVVTKDETTIVEGAGDSDAIAGRVAQIRTEIENSDSDYDRE 360

Query: 361 EKLQERLXXXXXXXXXXXXXXXTEVELKERKHRIEDAVRNXXXXXXXXXXXXXXXXXXXX 420
           EKLQERLTEVELKERKHRIEDAVRN
Sbjct: 361 EKLQERLAKLAGGVAVIKAGAATEVELKERKHRIEDAVRNAKAAVEEGIVAGGGVAFLQS 420
Query: 362  KLQERLXXXXXXXXXXXXXXXTEVELKERKHRIEDAVRNXXXXXXXXXXXXXXXXXXXX 421
            KLQERLTEVELKERKHRIEDAVRN
Sbjct: 361  KLQERLAKLAGGVAVIKAGAATEVELKERKHRIEDAVRNAKAAVEEGIVAGGGVALLHAI 420

Query: 421 XXXXDELKLTGDEATGANIVRVALSAPLKQIAFNAGLEPGVVAEKVSNLEAGHGLNADSG 480
             D+ KL GDEATGANIVRVALSAPLKQIAFNAGLEPGV+AEKVSNL AG GLNA +
Sbjct: 421 VPALDDFKLEGDEATGANIVRVALSAPLKQIAFNAGLEPGVLAEKVSNLPAGQGLNAQTN 480
Query: 422  XXXDELKLTGDEATGANIVRVALSAPLKQIAFNAGLEPGVVAEKVSNLEAGHGLNADSGE 481
             DELKL G+EATGANIVRVAL APLKQIAFN GLEPGVVAEKV N  AG GLNA +GE
Sbjct: 421  PALDELKLEGEEATGANIVRVALEAPLKQIAFNGGLEPGVVAEKVRNSPAGTGLNAATGE 480

Query: 481 EYEDLLAAGVADPVKVTRSALQNAASIAALFLTTEAVVADKPEKA-AAPAGDPTGGMGGM 539
           E EDLLAAGVADPVKVTRSALQNAASIAALFLTTEAVVADKPEKA AAPA MGGM
Sbjct: 481 EDEDLLAAGVADPVKVTRSALQNAASIAALFLTTEAVVADKPEKASAAPATGHRFKMGGM 540
Query: 482  YEDLLAAGVADPVKVTRSALQNAASIAALFLTTEAVVADKPEKAAAPAGDPTGGMGGMDF 541
            YEDLL AG+ADPVKVTRSALQNAASIA LFLTTEAVVADKPEKAAAPAGDPTGGMGGMDF
Sbjct: 481  YEDLLKAGIADPVKVTRSALQNAASIAGLFLTTEAVVADKPEKAAAPAGDPTGGMGGMDF 540

Query: 540 DF 541
           DF
Sbjct: 541 DF 542
```

Table 1/5: Transkriptase alpha chain of *N. farcinica* RPOA_NOCFA (Q5Z1K9)

| Description | SwissProt Accession # | Organism |
|---|---|---|
| _RHOSR | Q0S3E7 | *Rhodococcus sp. strain RHA1* |
| _MYCSS | Q1BD08 | *Mycobacterium sp. strain MCS* |
| _9MYCO | Q1T8C7 | *Mycobacterium sp. KMS* |
| _9MYCO | Q1TR19 | *Mycobacterium sp. JLS* |
| _MYCFV | Q27E10 | *Mycobacterium flavescens PYR-GCK* |
| _MYCVN | Q263M4 | *Mycobacterium vanbaalenii PYR-1* |
| RPOA_MYCTU | P66701 | *Mycobacterium tuberculosis* |
| RPOA_MYCBO | P66702 | *Mycobacterium bovis* |
| RPOA_MYCPA | Q73S43 | *Mycobacterium paratuberculosis* |
| RPOA_MYCLE | Q9X798 | *Mycobacterium leprae* |

Table 1/6: Enolase of *N. farcinica* ENO_NOCFA (Q5YQ30)

| Description | SwissProt Accession # | Organism |
|---|---|---|
| _RHOSR | Q0S4I1 | *Rhodococcus sp. strain RHA1* |
| _MYCSS | Q1B439 | *Mycobacterium sp. strain MCS* |
| _9MYCO | Q1TAN9 | *Mycobacterium sp. KMS* |
| _9MYCO | Q1TX18 | *Mycobacterium sp. JLS* |
| _MYCFV | Q275H2 | *Mycobacterium flavescens PYR-GCK* |
| ENO_MYCPA | Q741U7 | *Mycobacterium vanbaalenii PYR-1* |
| _MYCVN | Q267Y1 | *Mycobacterium tuberculosis* |
| ENO_MYCBO | Q7U0U6 | *Mycobacterium bovis* |
| ENO_MYCTU | P96377 | *Mycobacterium tuberculosis* |
| ENO_MYCLE | Q9CD42 | *Mycobacterium leprae* |

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## ARABIC SUMMARY

### إلتهاب الصدر المسبب بالنوكارديا و مشابهته لمرض الدرن (دراسة باكتيرية و بروتيومية)

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هدفت هذه الدراسة لمعرفة معدل حدوث الاصابة بالنوكارديا عند المرضى السودانيين المشتبه في اصابتهم بالسل الرئوي , وكذلك لمعرفة البروتينات المنتجة من النوكارديا التي سبق عزلها من مرضى تم تشخيصهم تشخيصا خاطئا على انهم مصابون بالسل الرئوي المقاوم للعلاج بالعقاقير المعروفة.

من اصل 329 مريض باصابات رئوية مختلفة تم استقطابهم لهذه الدراسة تم اخذ عينات تفاف لفحصها بتقنية صبغة الزيل نلسون ثم تم زرع كل عينة في انبوبين من وسط لونستين جنسن بـ 20 ميكروليتر من عينات التفاف المعادلة . تم تحضين المزارع البكتيرية في درجة حرارة 37˚ م لمدة 8 اسابيع. تم التشخيص الظاهري للبكتريا ومن ثم التعرف على البروتينات باستخدام تقنية جل الاكرايلاميد الثنائي الاتجاه لتحليل بروتينات عزلات النوكارديا ومقارنتها بعزلات بكتريا السل القياسية للبحث عن البروتينات المشابهة في قاعدة البيانات العالمية.

عشرة عزلات اظهرت نموا سريعا في خلال يومين الى ثلاثة ايام. اكدت التجارب التقليدية انتماء هذه العزلات الى عائلة النوكارديا بينما أظهرت نتائج تحليل البروتين ان هذه العزلات متشابهة في ما بينها في محتواها البروتيني وذات علاقة واضحة ببروتينات بكتيريا السل.

مما سبق يمكن استنتاج حدوث النوكارديا بمعدلات مقدرة (3.9%) معطية اعراضا سريرية مشابهة لتلك التي تحدث نتيجة للاصابة ببكتريا السل ربما بسبب تشابه البروتينات . وعليه ترجح هذه المعطيات حدوث النوكارديا لدى المرضى الذين يعانون من اصابة الرئتين المزمنة . وعليه من الضروري ان ينتبه الأطباء للمعالجون لهذا الاحتمال خصوصا عندما لا يستجيب المريض لأدوية السل التقليدية وذلك تفاديا للتشخيص الخاطئ ، أي حتى لا تشخص الاصابة بالنوكارديا الرئوية كاصابة ببكتريا السل المقاومة للعقاقير.