Actinomyces species isolated from breast infections

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ABSTRACT

Actinomycosis is a chronic infection caused by *Actinomyces* species characterized by abscess formation, tissue fibrosis, and draining sinuses. The spectrum of infections caused by *Actinomyces* species ranges from classical invasive Actinomycosis to a less invasive form of superficial skin and soft tissue infection. We present a review detailing all *Actinomyces* species isolated from breast infections in NHS Lothian between 2005 and 2013, *Actinomyces* species isolated from breast infections referred to the UK Anaerobe Reference Unit between 1988 and 2014 and cases describing *Actinomyces* breast infections published in the medical literature since 1994. *Actinomyces* species are fastidious organisms which can be difficult to identify and are likely to be under ascertained as a cause of breast infections. Due to improved diagnostic methods they are increasingly associated with chronic, recurrent breast infections and may play a more significant role in these infections than has previously been appreciated.

Keywords

Actinomycosis, Actinomyces, diphtheroids, breast infection, breast abscess.

INTRODUCTION

Actinomycosis is a chronic, invasive, progressive and often relapsing granulomatous infection caused by Gram-positive, facultatively anaerobic rod shaped bacteria belonging to the genus *Actinomyces*. Classical Actinomycosis in humans is typically caused by *Actinomyces israelii* and is characterized by deep invasive abscess formation, tissue fibrosis and draining sinuses affecting cervicofacial, thoracic, abdominopelvic areas (1). A number of more recently described *Actinomyces* species and Actinomyces-like organisms have been associated with less invasive superficial soft tissue infections and are isolated from abscesses at various anatomical sites (2,3,4).

Breast infections are frequently encountered in primary care and breast clinic settings. They can occur in the parenchyma of the breast or the overlying skin and may be in lactating or non-lactating breasts (5). Lactating breast infections are usually caused by *Staphylococcus aureus* (6). The microbial etiology of non-lactating breast infections, particularly those which are chronic or recurrent, is more variable, often polymicrobial and predominantly anaerobic (5,7). A study has demonstrated that when culture methods are used which enhance recovery of fastidious anaerobic organisms, almost 25% of recurrent breast abscesses (8 out of 33 patients) and 10% of primary breast abscesses (2 out of 19 patients) isolated *Actinomyces* species from non-puerperal breast infections (7). However, primary *Actinomyces* infection of the breast, first described by Ammentorp in 1893 (8), is generally considered to be rare. A clinical review from 1994 reported 19 cases described in the English language literature (9). Diagnosis of *Actinomyces* breast infection was often made following surgical intervention although the

method of diagnosis was not specified. Duration of symptoms was reported to range from 1-8 years, with two thirds of the patients presenting with recurrent abscesses. The remaining third of the patients were reported to have examination findings suggestive of malignancy. Most of the patients received extensive surgical treatment, with 11 patients undergoing a mastectomy, presumably for management of infection as only 3 were performed on patients with suspected malignancy. Subsequent to this review, case reports and studies have been published describing a further 27 breast infections caused by *Actinomyces* species (table 3).

Actinomyces breast infection is likely to be under ascertained in routine clinical practice, as these fastidious organisms are notoriously difficult and laborious to identify using conventional laboratory methods (2,3,4). Subsequently there is a risk that cultures of *Actinomyces* species are simply identified morphologically as “diphtheroids” and dismissed as skin commensals, even when grown from an abscess sample. However, new methods of identification such as matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) allow rapid and reliable identification of many bacteria, including *Actinomyces*-like organisms (10,11,12). MALDI-TOF MS and similar technologies are increasingly being adopted by routine diagnostic laboratories worldwide (13).

We present a series describing all cases of *Actinomyces* species isolated from breast infections at the Edinburgh Breast Unit over an 8 year period from 2005-2013. Further to this we include data from *Actinomyces* species isolated from breast samples which were referred to the Anaerobe Reference Unit, Cardiff between 1988 and 2013. We then summarize the

findings of case reports describing *Actinomyces* species causing breast infection published since the review from 1994 (9).

MATERIALS AND METHODS

Bacterial isolates. Review of electronic records of breast fluid aspirates received between 2005 and 2013 at the microbiology laboratory at the Royal Infirmary of Edinburgh identified eleven cases of breast infections with *Actinomyces* species and one case with the Actinomyces-like organism *Actinobaculum schaali*. Specimens were collected either as pus in sterile containers or on swabs (Stewarts media) and routinely transported to the laboratory. Fastidious anaerobic agar with horse blood (not pre-reduced) was used for culture, incubated in an anaerobic cabinet (80% nitrogen, 10% $C0_2$, 10% hydrogen) for at least 48 hours. Until 2011, Gram-positive rods were identified using biochemical methods, generally API Coryne (bioMérieux). From 2011 onwards isolates were identified using MALDI-TOF MS (Bruker Daltonics).

The UK Anaerobe Reference Unit in Cardiff, Wales (UKARU) provided details of *Actinomyces* isolates referred to them from hospitals throughout the UK between 1988 and 2014, where the source stated on the request form was 'breast' (abscess/fluid/wound).

MALDI-TOF mass spectrometry. MALDI-TOF MS identification was carried out using a Bruker MicroFlex LT mass spectrometer (Bruker Daltonics) and Bruker FlexControl V3.3 software. Isolates were analyzed using a formic acid-based direct, on-plate preparation method. A thin smear of organism was

applied to a target plate using a cocktail stick, allowed to dry and then 1µl of 100% formic acid was placed on top and allowed to dry. This mixture was overlain with 1µl of matrix solution (cyano-4-hydroxycinnamic acid) and allowed to dry prior to analysis using the MALDI Biotyper. Manufacturer-recommended cutoff scores were used for identification, with scores of >2.000 indicating identification to the species level, scores between 1.700 and 1.999 indicating identification to the genus level, and scores of <1.700 indicating no identification.

Molecular identification. Definitive molecular identification was by 16S sequencing using the following method. 16S rDNA was extracted using a chelex resin/boiling method and amplified by PCR using the universal primers pA & pH'. After purification (Qiaquick PCR purification kit #28106, Qiagen), a second PCR reaction was performed using a primer internal to the initially amplified region ('kk') and dye-terminated nucleotides (Big Dye 3.1 Terminator Ready Reaction kit). After a second purification step, the sequence of bases was detected by size / dye terminator of the resulting DNA fragments (ABI, 3100). The sequences were compared locally with those of other bacteria (ARU bespoke database, Bionumerics, Applied Maths) or with those listed in international databases (NCBI, BLAST®), with 16S rDNA homology of >97% used to determine bacterial species.

Clinical review. Paper and electronic patient records of cases were reviewed for information on; age, smoking history, diabetes, nipple piercing, steroid use and whether the patient was lactating at the time of infection. The number of times the patient came into contact with the Edinburgh breast unit was recorded, along with examination findings and management received.

Information was collected on GP clinic appointments for breast infections, along with the type, duration and number of antibiotic courses for breast infection in the community.

Literary review of published cases was completed on Pubmed and Ovid databases using the keywords: actinomyces, actinomycosis, breast, infection, abscess. Cases of Actinomyces infection of the breast published with clinical descriptions between 1994-2013 are detailed in table 3. Two cases from 1987 not included in the 1994 review (9) are included in this table.

RESULTS

Table 1 – Cases of *Actinomyces* species isolated from breast infections at the Edinburgh Breast Unit over an 8 year period from 2005-2013.

Over an 8 year period (2005-2013) we identified eleven cases of breast infections at our center caused by *Actinomyces* species and one case with the Actinomyces-like organism *Actinobaculum schaali*. The predominant *Actinomyces* species isolated from our subgroup of patients were *Actinomyces europaeus* (n=5), *A. neuii* (n=3) and *A. radingae* (n=3). Identification using MALDI-TOF MS was attempted for 11 isolates and all of these correlated at species level with the definitive molecular identification, with p scores ranging between 1.779 and 2.331. Co-infecting organisms were present in half of these cases (n=6), usually unidentified ‘anaerobes’. Ten out of 12 cases (83%) had chronic, recurrent infection ranging from 2-8 (mean 2.8) episodes, some over many years. Three patients had hidradenitis

suppurativa, 6 patients were smokers and 4 were diabetic with 3 of these patients having a combination of risk factors. No patients in our cohort had a lactational breast abscess and there was no record of any patients having had a nipple piercing.

Case number 5 in particular highlights the difficulties associated with diagnosing and managing *Actinomyces* breast infections. This patient had 7 episodes of breast infection and abscess formation over a 10 year period treated with short antibiotic courses. Cultures of aspirated abscess material repeatedly failed to grow organisms or were reported to grow "diphtheroids" of uncertain significance. Following a positive growth of *A. radingae* she received a 3 month course of antibiotics and has since had no further relapses (almost 2 years later).

Table 2 – *Actinomyces* species isolated from breast infections referred to the UK Anaerobe Reference Unit from UK hospitals 1988-2014.

Over a 26 year period (1988-2014), 61 isolates identified as *Actinomyces* species from breast infections were referred to the UK Anaerobe Reference Unit (UKARU) from UK hospitals. Although not considered 'true anaerobes', the UKARU has developed extensive expertise over many years regarding *Actinomyces* species. This was driven largely by a referral demand from UK users for advanced identification of clinically relevant isolates initially categorized as 'anaerobic gram positive rods' or 'anaerobic coryneforms'. It is likely that the cases listed here represent only a small proportion of UK cases,

as referral of isolates to the unit is not mandatory. Unfortunately a further limitation of the referral process is that clinical information is not available for many of these cases, however a small number (n=5) state either 'recurrent' or 'previous breast abscess'. One case worthy of particular mention states 'recurrent breast abscess for 11 years' from which *A. radingae* was isolated.

Table 3 – Published cases of *Actinomyces* species isolated from breast infections reported with clinical details since 1994 (ref 14-28).

Fifteen cases of *Actinomyces* breast infection were identified on literature review between 1994 and 2013, with another 12 cases (7,14) found prior to 1994, not included in the Jain et al review (9). This paper therefore reports an additional 27 published cases of *Actinomyces* breast infection to the 19 reported in 1994, although clinical details are only available for 17 of these 27 cases. There was no clearly predominant *Actinomyces* species. Five cases reported co-infecting anaerobes (17,24) and one case S*taphylococcus aureus* (20), with no mention of co-infecting organisms in 11 of 17 cases.
Seven cases were recurrent infections and a range of different treatment combinations were required to reach abscess resolution. This ranged from 2-3 weeks of oral antibiotics, to incision and drainage with prolonged antibiotics for 2-6 months, with the most extreme being that of tumorectomy of the breast (22). As with our cohort of patients, prior to the diagnosis of *Actinomyces* breast infection, some patients had been repeatedly treated without success (14,17,20).

Table 4 – Combined number and species of *Actinomyces* breast infections from tables 1-3 and from reference 7.

* Two Lothian cases had two different Actinomyces sp. isolated

† Isolates referred from Lothian are removed from this column to avoid double counting

‡ Published cases include 10 cases from ref 7 with no clinical details

The *Actinomyces* species most commonly isolated from breast infections according to this combined table (n=102) are *A. neuii* (n=19), *A. europaeus* (n=18), *A. turicensis (*n=16), *A. radingae* (n=15) and *A. odontolyticus* (n=10). These mostly belong to the group of *Actinomyces* species generally considered to be less invasive, although it is noteworthy that in the Lothian and UK cohorts the cases with the greatest number of relapses all isolated *A. radingae*. The distribution of *Actinomyces* species broadly reflects previous findings regarding superficial *Actinomyces* soft tissue infections (2,3,4), although these studies did not look specifically at breast infections.

Within the NHS Lothian and the published cases 48% (n=14) presented clinically with an abscess, 33% (n=9) presented with a breast mass, 10% (n=3) with a fistula and 7% (n=2) presented with periductal mastitis. There was an average of 2.8 episodes of infection per patient in the NHS Lothian cases. Within the published cases, excluding case 1 who had numerous yearly episodes of recurrent infection for 23 years, there was an average of 1.5 episodes of *Actinomyces* breast infection per patient.

DISCUSSION

Humans and animals are the natural reservoirs of *Actinomyces* species, which until recently have not been found to exist freely in nature (29). Their normal habitat is the mucosal membranes of the oropharynx, gastrointestinal tract and female genital tract. They are inherently low virulent and may rely on the presence of co-pathogens, such as anaerobic bacteria, to enhance pathogenicity (1). Disruption of the mucosal barrier is the usual precursor to infection with *Actinomyces* species and in the breast, the ductal system may serve as a portal of entry. Actinomycosis of the breast usually presents as a chronic, recurrent abscess which in some cases can be difficult to distinguish from inflammatory carcinoma (9,15,26). Fistulas and purulent or bloody discharge from sinuses may occur which may discharge “sulfur” granules (26). In advanced prolonged cases, fibrosis with architectural distortion of the breast tissue is present on mammography (15).

The pathogenesis and true pathogenic role of various *Actinomyces* species isolated from breast infections and the treatment required for this has not been clearly defined. This is further complicated by the uncertain etiology of different types of chronic abscess-forming inflammatory conditions involving the breast, from which *Actinomyces*-like organisms can be isolated, such as granulomatous lobular mastitis, hidradenitis suppurativa and periductal mastitis. Granulomatous lobular mastitis presents as a peripheral inflammatory mass which may simulate malignancy or infection. Patients with this condition often develop multiple and recurrent abscesses. It has been

279 suggested that the *Corynebacterium* spp play a part in this condition, (30) but
280 antibiotics effective against these organisms rarely lead to resolution of
281 disease and thus they may not have a major etiological role. Hidradenitis
282 suppurativa is an inflammatory disease of unclear etiology which commonly
283 affects the axilla and groin and can also affect the skin of the lower half of the
284 breast, resulting in recurrent episodes of abscess formation (31). Recent
285 evidence suggests that anaerobic actinomycetes may be involved in the
286 disease process, especially when lesions are more severe (32). Periductal
287 mastitis is a condition linked to cigarette smoking (33) in which the subareolar
288 ducts are damaged and become infected, often by anaerobic bacteria (34).
289 Women may present with subareolar inflammation, abscesses and fistulas
290 (35). Smoking has consistently been identified as a risk factor for primary
291 breast abscess and its recurrence (5,35,36). Other factors, such as diabetes
292 mellitus, obesity, African-American origin and nipple piercing have less
293 consistently been associated with breast abscesses (5,35).
294 Despite finding 12 cases over 8 years at our center, which is
295 comparable to the number of cases described in the medical literature over
296 the same time period, we suspect that there were many missed
297 identifications. During the 8 year study period, we found another 15 cases in
298 Lothian where potential Actinomyces-like organisms were isolated from
299 recurrent breast abscesses, but further identification was not attempted and a
300 report was sent out describing ‘diphtheroids’ of doubtful or uncertain
301 significance. In addition, 4 out of our 12 culture positive cases had previous
302 samples with isolates of potential Actinomyces-like organisms reported as
303 ‘diphtheroids’ of doubtful significance. This supports the assumption that

Actinomyces breast infections may easily go undiagnosed in routine clinical practice. *Actinomyces* species are slow to grow and notoriously difficult to identify using conventional laboratory methods, often requiring reference laboratory referral for reliable identification. When *Actinomyces* species do grow on culture they can resemble other diphtheroid-like Gram-positive rods, such as *Corynebacterium* species, many of which are considered to be part of normal skin flora. *Actinomyces* species which are isolated from breast abscess samples may therefore be presumptively identified in the laboratory as 'diphtheroids' based on their morphology and reported as 'diphtheroids' of doubtful or uncertain significance. However, laboratories are increasingly adopting new methods of identification, such as MALDI-TOF MS (13), which allow rapid and increasingly reliable identification of this problematic group of organisms (10,11,12). Indeed, most of the cases in Lothian were identified after 2012, which is shortly after our laboratory started using MALDI-TOF MS. With 10 cases diagnosed in 2 years of using MALDI-TOF MS compared to 2 cases over 7 years without MALDI-TOF MS, it is clear that ease of identification is a major factor in the increased recognition of *Actinomyces* breast infections in our clinical setting. The Anaerobe Reference Unit (ARU) has seen a similar increase in the number of isolates referred to them, with more isolates (n=26) referred to them over the last 3 years of the recorded period than had been referred over the first 20 years (n=25). Based on information from referring laboratories, this increase is almost certainly driven by an improvement in the identification of *Actinomyces* species due to increased use of MALDI-TOF MS. Subsequently, UK laboratories unfamiliar

with these organisms refer them to the ARU for confirmation of identification, susceptibility testing and clinical advice.

Our results support previous findings that *Actinomyces* species can be reliably identified using MALDI-TOF MS (10,11,12), with all 12 of our tested isolates identified to species level, as confirmed by molecular testing. Five isolates were correctly identified to species level by MALDI-TOF MS despite identification scores only reaching genus level confidence ($p<2.0$). This is in keeping with recent evidence suggesting that the cut-off for species level identification could be reassessed and perhaps lowered to $p>=1.7$ for this group of organisms (10,12).

We have modified the approach to how organisms from breast samples are identified in Lothian. Breast abscess samples now receive anaerobic incubation for 5 days, along with prolonged *Actinomyces* cultures when clinical details mention chronic or recurring infection. Any Gram-positive bacillus growing from a breast abscess sample is identified using MALDI-TOF MS and should no longer be reported as a “diphtheroid” of uncertain significance without an attempt being made to identify the organism.

The primary management of breast abscess is drainage, along with antibiotic therapy appropriate for the underlying cause of the abscess (31). When *Actinomyces* species are isolated, longer courses of antibiotics should be considered. Treatment of classical, invasive Actinomycosis, typically caused by *Actinomyces israelii* and to a lesser extent *A. gerencseriae*, *A. meyeri*, *A. odontolyticus* and *A. viscosus/naeslundii* (2,4,37), involves prolonged antibiotic therapy. Textbooks commonly advise 2-6 weeks of intravenous penicillin followed by 6-12 months of oral penicillin or amoxicillin

(38). However, there is evidence that shorter antibiotic courses of under 3 months may be sufficient in some cases (39), particularly those caused by less invasive *Actinomyces* species, such as *A. europaeus*, *A. funkei*, *A. neuii*, *A. radingae* and *A. turicensis* (2,3,4,37,40). Even shorter 7-14 day courses of oral antibiotics are typically used when treating breast infections, but this is likely to be insufficient for Actinomyces associated breast infections and longer courses, in addition to surgical drainage, may be required to prevent recurrences. *Actinomyces* species are susceptible to many beta-lactam antibiotics, with penicillin and amoxicillin generally regarded as first choice options (38,41). However, due to the common presence of co-infecting, beta-lactamase producing organisms, treatment options should ideally include beta-lactamase stable antibiotics, such as amoxicillin plus clavulanic acid (41), at least for the initial 2 weeks of treatment. Alternative agents for patients with penicillin allergy could include doxycycline or clindamycin, although there is less evidence for their efficacy (38). We suggest at least 6 weeks of antimicrobial treatment for extensive infections involving *Actinomyces* species or in cases where recurrences have occurred. Although some recurrent cases of Actinomyces breast infections seem to have benefited from this, it is not clear whether a longer course of antimicrobial in the first instance would have prevented relapses in these cases.

CONCLUSION

Actinomyces associated breast infections are problematic, difficult to diagnose and difficult to treat. They are increasingly recognized in clinical practice, most likely due to a combination of increased awareness and improved diagnostic methods. Further studies are required to clarify the pathogenic role of *Actinomyces* species in various inflammatory conditions which involve the breast, such as periductal mastitis, hidradenitis suppurativa and granulomatous mastitis. These conditions all present with clinical features similar to those seen in Actinomyces breast infections. Taking into account the fastidious nature of *Actinomyces* species, it is quite possible that anaerobic actinomycetes are present significantly more frequently than they are found in these conditions. In particular, it is important to clarify whether longer initial courses of effective antibiotic treatment may prevent recurrences and radical surgery when *Actinomyces* species are isolated in association with these conditions.

REFERENCES

1. **Smego RA, Foglia G.** 1998. *Actinomycosis*. Clin Infect Dis **26**:1255–1263.
2. **Clarridge JE, Zhang Q.** 2002. Genotypic Diversity of Clinical *Actinomyces* Species: Phenotype, Source and Disease Correlation among Genospecies. J Clin Microbiol **40**(9):3442-3448.
3. **Sabbe LJ, Van de Merwe D, Schouls L, Bergmans A, Vaneechoutte M, Vandamme P.** 1999. Clinical spectrum of infections due to newly

described *Actinomyces* species *A. turicensis, A. radingae* and *A. europaeus.* J Clin Microbiol **37**:8–13.

4. **Hall V, Talbot PR, Stubbs SL, Duerden BI**. 2001. Identification of clinical isolates of *Actinomyces* species by amplified 16S ribosomal DNA restriction analysis. J Clin Microbiol **39**:3555–3562.
5. **Bharat A, Gao f, Aft R. L, Gillanders W. E, Timothy J. Eberlein T.J, Margenthaler J. A.** 2009. Predictors of Primary Breast Abscesses and Recurrence. World J Surg **33**:2582–2586.
6. **Dabbas N, Chand M, Pallett A, Royle G.T, Sainsbury R.** 2010. Have the Organisms that Cause Breast Abscess Changed With Time? Implications for Appropriate Antibiotic Usage in Primary and Secondary Care. Breast J **16**(4):412–415.
7. **Edmiston CE, Walker AP, Krepel CJ, Gohr C**. 1990. The Nonpuerperal Breast Infection: Aerobic and Anaerobic Microbial Recovery from Acute and Chronic Disease. J. Infect. Dis **162**:695-699.
8. **Lloyd Davies JA**. 1951. Primary *actinomycosis* of the breast. Br J Surg **38**:378–381.
9. **Jain BK, Sehgal VN, Jagdish S, Ratnakar C, Smile SR**. 1994. Primary *actinomycosis* of the breast: a clinical review and a case report. J Dermatol **21**:497–500.
10. **Schmitt BH, Scott A. Cunningham AL, Dailey DR, Gustafson RP**. 2013. Identification of Anaerobic Bacteria by Bruker Biotyper Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry with On-Plate Formic Acid Preparation. J. Clin. Microbiol **51**(3):782-786.

11. **Ng LSY, Sim JHC, Eng LC, Menon S, Tan TY**. 2012. Comparison of phenotypic methods and matrix-assisted laser desorption ionisation time-of-flight mass spectrometry for the identification of aero-tolerant *Actinomyces sp.* isolated from soft-tissue infections. Eur J Clin Microbiol Infect Dis **31**:1749–1752.
12. **Barberis C, Almuzara M, Join-Lambert O, Ramirez MS, Famiglietti A, Vay C.** 2014. Comparison of the Bruker MALDI-TOF Mass Spectrometry System and Conventional Phenotypic Methods for Identification of Gram-Positive Rods. *PLoS ONE* **9**(9): e106303. doi: 10.1371/journal.pone.0106303.
13. **Patel R**. 2013. Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry in Clinical Microbiology. Clin. Infect. Dis **57**(4):564–572.
14. **Allen JN.** 1987. *Actinomyces meyerii breast abscess* (letter). AM J Med **83**:186-187.
15. **De Barros N, Issa FKK, Barros ACSD, D'Ávila MS, Nisida AC, Chammas MC, Pinotti JA, Cerri GG.** 2000. Imaging of Primary *Actinomycosis* of the Breast. Am J Roentgenol **174**(6):1784-1786.
16. **Capobianco G, Dessole S, Becchere MP, Profili S, Cosmi E, Cherchi PL, Meloni GB.** 2005. A rare case of primary *actinomycosis* of the breast caused by *Actinomyces viscosus:* diagnosis by fine-needle aspiration cytology under ultrasound guidance. Breast J **11**(1): 57-59.

17. **Attar KH, Waghorn D, Lyons M, Cunnick G**. 2007. Rare Species of *Actinomyces* as Causative Pathogens in Breast Abscess. Breast J **13**(5):501–505.
18. **Lacoste C, Klijanienko J, Escande M-C, Jammet P, Nos C.** 2009. Breast *Actinomyces neuii* abscess simulating primary malignancy: A case diagnosed by fine-needle aspiration. Diagn Cytopathol **37**(4):311–312.
19. **Akhlaghi M, Ghazvini RD.** 2009. Clinical Presentation of Primary *Actinomycosis* of the Breast. Breast J **15**(1):102–103.
20. **Al-Niaimi F, Patel A, Blessing K, Fox R, Burden AD**. 2010. Cutaneous *actinomycosis* presenting as chronic mastitis Clin Exp Dermatol **35**(2):149–151.
21. **Salmasi A, Asgari M, Khodadadi N, Rezaee A.** 2010. Primary *Actinomycosis* of the Breast Presenting as a Breast Mass. Breast Care (Basel) **5**(2):105–107.
22. **Roustan A, Al nakib M, Boubli L.** 2010. Primary *actinomycosis* of the breast due to *Actinomyces neuii.* J Gynecol Obstet Biol Reprod **39**:64-67
23. **Gómez-Garcés JL, Burillo A, Gil Y**. 2010. Soft Tissue Infections Caused by *Actinomyces neuii*, a Rare Pathogen. J. Clin. Microbiol **48**(4):1508-1509.
24. **Silva WA, Pinheiro AM, Jahns B, Bögli-Stuber K, Droz S, Zimmerli S.** 2011. Breast abscess due to *Actinomyces europaeus*. Infection **39**(3):255-258.

25. **Arora B, Giri S,Arora DR.** 2012. *Actinomycosis* of breast-a case report. Int J Cur Res Rev **4**(20):85-88.
26. **Thambi R, Devi L, Sheeja S, Poothiode U.** 2012. Primary breast *actinomyces* simulating malignancy: A case diagnosed by fine-needle aspiration cytology. J Cytol **29**(3):197–199.
27. **Gupta C, Singh P, Bedi S, Kapur K.** 2012. Primary *Actinomycosis* of the Breast Masquerading as Malignancy: Diagnosis by Fine Needle Aspiration Cytology. Breast Care (Basel) **7**(2):153–154.
28. **Rich BS, Angeles C, Barie PS**. 2013. *Actinomyces odontolyticus:* Breast Abscess. Surgical Infections **14**(3):331-332
29. **Rao JU, Rash BR, Nobre MF, da Costa MS, Rainey FR, Moe WM***. 2012. Actinomyces naturae* sp. nov., the first *Actinomyces* sp. isolated from a non-human or animal source. Antonie Van Leeuwenhoek. **101**(1):155-168.
30. **Paviour S, Musaad S, Roberts S, Taylor G, Taylor S, Shore K, Lang S, Holland D.** 2002. *Corynebacterium* Species Isolated from Patients with Mastitis*.* Clin Infect Dis **35**:1434–1440
31. **Dixon MJ, Khan LR.** 2011. Clinical Review: Treatment of breast infection. BMJ **342**:d396.
32. **Guet-Revillet H, Coignard-Biehler H, Jais JP, Quesne G, Frapy E, Poirée S, Le Guern A-S, Le Fleche-Mateos A, Hovnanian A, Consigny P-H, Lortholary O, Nassif X, Nassif A, Join-Lambert.** 2014. Bacterial pathogens associated with hidradenitis suppurativa, France. Emerg Infect Dis **20**(12):1990–1998.

33. **Bundred NJ, Dover MS, Aluwihare N, Faragher EB, Morrison JM.** 1993. Smoking and periductal mastitis. BMJ **307**:772-773.

34. **Bundred NJ, Dixon JM, Lumsden AB, Radford D, Hood J, Miles RS, Chetty U, Forrest AP**. 1985. Are the lesions of duct ectasia sterile? Br J Surg **72**(10):844-845.

35. **Dixon JM.** 1998. Periductal mastitis and duct ectasia: an update. Breast J **7**(3):128-130

36. **Gollapalli V, Liao J, Dudakovic A, Sugg SL, Scott-Conner CEH, Weigel R.** 2010. Risk Factors for Development and Recurrence of Primary Breast Abscesses. J Am Coll Surg **211**(1):41-48.

37. **Hall V.** 2008. *Actinomyces*—gathering evidence of human colonization and infection. Anaerobe **14**:1–7.

38. **Russo TA**. 2009. Agents of *actinomycosis*: chapter 255. *In* Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 7th ed. Churchill Livingstone, Elsevier.

39. **Sudhakar SS, Ross JJ.** 2004. Short-Term Treatment of *Actinomycosis:* Two Cases and a Review. Clin Infect Dis **38**:444–7

40. **Von Graevenitz A.** 2011. *Actinomyces neuii:* review of an unusual infectious agent. Infection **39**:97–100.

41. **Smith AJ, Hall V, Thakker B, Gemmell CG.** 2005. Antimicrobial susceptibility testing of *Actinomyces* species with 12 antimicrobial agents. J Antimicrob Chemother **56**:407-40

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Table 1 Cases of Actinomyces species isolated from breast infections at the Edinburgh Breast Unit over an 8 year period from 2005-2013

Patient	Year	Age (years) / sex	Risk factor(s) / PMH	Type of breast infection	MALDI-TOF identification (p=score)	Molecular identification (16S sequencing)	Co-infecting organisms	No. of infections	Previous potential Actinomyces isolate not identified as such by laboratory	Comments on outcome (incl antibiotic treatment, surgery, resolution)
1	2013	36 F	Nil	Left breast abscess	*Actinomyces radingae* (p=2.0234) *Actinomyces europaeus* (p=1.972)	*Actinomyces radingae* and *Actinomyces europaeus*	Yes (*Peptoniphilus* sp.)	1	No	Good response to drainage and clindamycin.
2	2013	52 F	Diabetes	Right breast infra-mammary fold abscess	*Actinomyces europaeus* (p=1.779)	*Actinomyces europaeus*	No	1	No	Abscess aspirated and patient treated with flucloxacillin. Complete resolution 3 months later.
3	2013	36 F	Nil	Left breast abscess	*Actinomyces odontolyticus* (p=2.006)	*Actinomyces odontolyticus*	No	2	First isolate initially reported as "Diphtheroid" of doubtful significance.	Chronic breast lump slowly increasing in size over 8 months, initially investigated as potential malignancy. Core biopsy revealed changes consistent with chronic abscess and purulent aspirate grew *Actinomyces odontolyticus*.
4	2013	26 F	Nil	Left breast abscess (infected epidermoid cyst)	*Actinomyces neuii* (p=2.3314)	*Actinomyces neuii*	No	1	No	Initial partial response to amoxicillin-clavulanic acid which was changed to ciprofloxacin due to intolerance. Apparent relapse which settled after 6 weeks of amoxicillin. Residual mass excised, pathology showed epidermoid cyst.
5	2013	41 F	Nil	Left breast abscess	*Actinomyces radingae* (p=2.0348)	*Actinomyces radingae*	Yes (multiple anaerobe species)	7 (over 10 years)	Yes (sample from 2012 with "diphtheroids" and anaerobes).	Multiple recurrences of breast abscesses, with no growth on culture as patient was already on antibiotics. Sample from 2012 isolated "diphtheroids" and anaerobes. Patient's GP was advised to refer patient for aspiration before starting antibiotics if abscess recurred. This resulted in growth of *Actinomyces radingae* along with multiple anaerobe spp. Treated with drainage and amoxicillin-clavulanic acid and metronidazole for 2 weeks followed by 3 months of amoxicillin. No further recurrences almost 2 years later.
6	2013	19 F	Smoker / Hidradenitis suppurativa	Breast abscess	*Actinomyces* species (p=2.0186)	*Actinomyces* species (closest sequence *Actinomyces europaeus*)	No	1	No	Breast abscess drained. Patient treated with amoxicillin-clavulanic acid for 1 week with apparent resolution.

Table 1 Cases of Actinomyces species isolated from breast infections at the Edinburgh Breast Unit over an 8 year period from 2005-2013

Patient	Year	Age (years) / sex	Risk factor(s) / PMH	Type of breast infection	MALDI-TOF identification (p=score)	Molecular identification (16S sequencing)	Co-infecting organisms	No. of infections	Previous potential Actinomyces isolate not identified as such by laboratory	Comments on outcome (incl antibiotic treatment, surgery, resolution)
7	2012	34 F	Smoker	Left and right breast abscesses	*Actinomyces radingae* (p=1.829)	*Actinomyces radingae*	Yes (*Actinobaculum schaalii* and multiple anaerobe species)	8	No	Multiple recurrences of breast abscesses over a period of 12 months treated with aspirations and short courses of antibiotics (mostly combinations of flucloxacillin, amoxicillin-clavulanic acid and metronidazole). Referred for mammary fistula and total duct excision of right breast.
8	2012	46 F	Diabetes / Smoker / Hidradenitis suppurativa	Breast abscess	*Actinomyces neuii* (p=2.076)	*Actinomyces neuii*	No	2	Yes (sample from 2011 with "diphtheroids" and anaerobes).	Abscess drained and patient treated with antibiotics. Previous episode in 2011 treated with amoxicillin-clavulanic acid and metronidazole.
9	2012	38 F	Smoker	Breast abscess and periductal mastitis	*Actinobaculum schaalii* (p=not available)	*Actinobaculum schaalii*	Yes (*Streptococcus constellatus*)	4 (over 3 years)	Yes (sample from 2011 with "diphtheroids" and alpha-haem streptococci).	Recurrent left breat periductal mastitis and abscess, 4 episodes over 3 years. Treated with antibiotics and sometimes aspiration.
10	2011 - 2012	36 F	Diabetes / Smoker / Hidradenitis suppurativa	Left and right breast abscesses	*Actinomyces neuii* (p=1.921) and *Actinomyces europaeus* (p=<2.0)	*Actinomyces neuii* and *Actinomyces europaeus*	Yes (Anaerobes)	2	No	Two separate breast abscesses left and right breast 3 months apart. First episode treated with multiple 7 day courses of flucloxacillin and/or amoxicillin. Second episode treated with 3 months of amoxicillin. No recurrence 18 months later.
11	2005	43 F	Smoker	Left periductal mastitis	Not available. Sent to reference laboratory.	*Actinomyces europaeus*	Yes (Anaerobes)	2	Isolate identified as "*Corynebacterium* species" and sent to reference laboratory.	Two episodes of periductal mastitis in 1997-98, requiring aspiration (no microbiological data available) and 4 weeks of antibiotics. Presented again in 2005 with an abscess requiring drainage twice 4 weeks apart. Treated with 10 days of oral amoxicillin-clavulanic acid.
12	2005	59 F	Diabetes	Left breast abscess	Not available. Sent to reference laboratory.	*Actinomyces europaeus*	No	Unknown	Isolate identified as "*Corynebacterium* species" and sent to reference laboratory.	Information not available.

Table 2. Actinomyces species isolated from breast infections referred to

UK Anaerobic Reference Unit from UK hospitals 1988-2014

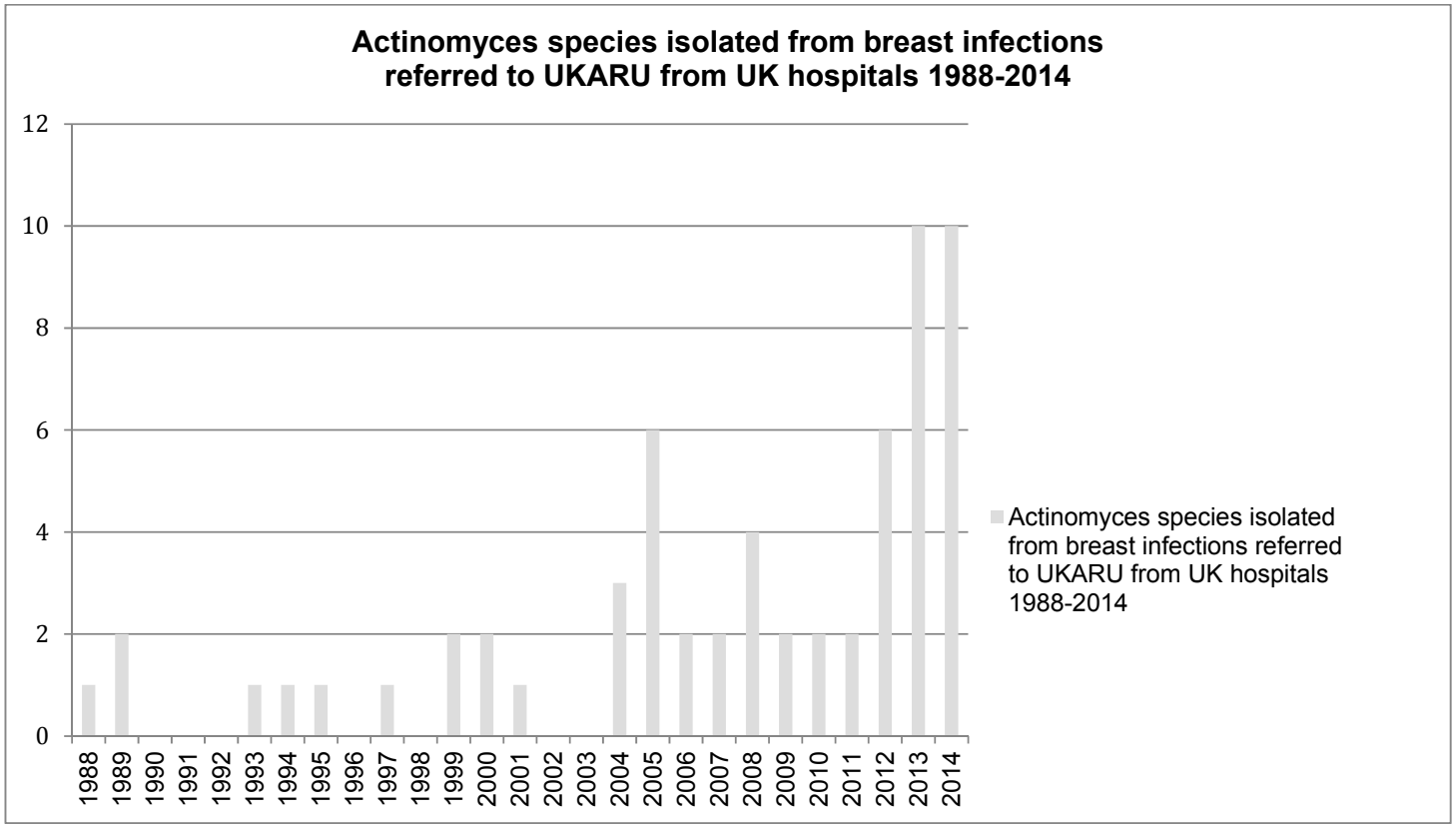

Table 3 - Published cases of Actinomyces species isolated from breast infections reported with clinical details since 1994

Patient	Country	Year	Age	Risk factor(s) / PMH	Clinical description	Organism	Method of identification	Co-infecting organisms	No. of infections	Comments on outcome (incl antibiotic treatment, surgery, resolution)	Ref
1	USA	1987	29	Severe peridental disease	Hard 5x4 cm left breast mass	*Actinomyces meyerii*	Not specified	Diverse anaerobes	1-3 episodes every year for 23 years	Surgical debridement with ampicillin, doxycyline for 4 months	14
2	USA	1987	36	Recurrent peridental abscesses	Hard 4x4 cm right breast mass	*Actinomyces meyerii*	Not specified	Diverse anaerobes	5 recurrences over 3 years	Surgical debridement with tetracycline, doxycycline for 4 months	14
3	Brazil	2000	66	Diabetes	5 year history of a mass in the left breast. Nipple discharge, cutaneous fistulas.	*Actinomyces* species	Histopathologic examination. Culture of abscess 4 years previously isolated *Actinomyces* sp.	None mentioned	2	Abscesses and fibrous tissue drained and resected. Responded to 2 months of IV penicillin followed by oral amoxicillin for 6 months.	15
4	Italy	2005	27	Nil	Unilateral right mastitis, palpable 5 cm nodular lump just beside the right areola.	*Actinomyces viscosus*	Culture positive. Biochemical tests.	None mentioned	1	One week of an unspecified antibiotic with no response. Then oral amoxicillin/clavulanic acid followed by surgical drainage and excision of the lesion with no further antibiotics. Resolution after 6 year follow up.	16
5	UK	2007	33	Bilateral nipple piercings removed 6 months prior to presentation. On 5mg prednisolone for ulcerative colitis, smoker.	3 week history of right breast pain, swelling, and offensive nipple discharge.	*Actinomyces turicensis*	Culture positive. Confirmed with 16S rDNA restriction analysis.	Mixed anaerobes	3	Aspiration and amoxicillin/clavulanic acid for 7 days. Worsening symptoms and three more attempts to aspirate over the following 2-week period. Incision and drainage with full excision of abscess wall, followed by ceftriaxone and oral metronidazole for 3 weeks. Complete resolution at follow up 8 weeks later.	17

Table 3 - Published cases of Actinomyces species isolated from breast infections reported with clinical details since 1994

Patient	Country	Year	Age	Risk factor(s) / PMH	Clinical description	Organism	Method of identification	Co-infecting organisms	No. of infections	Comments on outcome (incl antibiotic treatment, surgery, resolution)	Ref
6	UK	2007	38 (male)	Ex-smoker	Right axillary and left subareolar abscess with nipple discharge for 7 months. Left axillary abscess drained 18 months previously.	*Actinomyces radingae* (isolated from subareolar abscess)	Culture positive. Confirmed with 16S rDNA restriction analysis.	Heavy growth of anaerobes (from right axillary abscess)	3	Incision and drainage of abscesses. Oral amoxicillin and fusidic acid for 6 weeks. Recurrence of *A. radingae* followed by a prolonged course of oral ciprofloxacin and rifampicin with eventual resolution.	17
7	France	2009	48	Pregnant	Inflammatory breast mass 15 mm. Clinically and radiologically interpreted as carcinoma.	*Actinomyces neuii*	FNA showed granulomas. Culture positive. Confirmed with 'genetic amplification'.	None mentioned	1	Treated with 3 weeks of oral amoxicillin with resolution	18
8	Iran	2009	30	Nil	Few days history of sudden painful and swollen left breast with multiple fistula formation.	*Actinomyces israelii*	Morphology on culture	None mentioned	1	Responded to treatment with oral erythromycin for 6 months.	19
9	UK	2010	35	7 months post-partum	3 month history of tenderness and induration in the right breast	Not isolated on culture	Histopathologic examination	Staphylococcus aureus on skin swab	1	Treated with oral penicillin with little improvement. Further treatment with imipenem, coamoxiclav and metronidazole had little effect. Finally treated for over 12 months with oral clindamycin with resolution.	20
10	Iran	2010	48	Psychiatric problem	2 month history of non-tender mass in the left breast.	Not isolated on culture	Histopathologic examination	None mentioned	1	Treated with 4 weeks of intravenous penicillin, followed by oral amoxicillin for 4 months. Fully resolved at 2 year follow up.	21

Table 3 - Published cases of Actinomyces species isolated from breast infections reported with clinical details since 1994

Patient	Country	Year	Age	Risk factor(s) / PMH	Clinical description	Organism	Method of identification	Co-infecting organisms	No. of infections	Comments on outcome (incl antibiotic treatment, surgery, resolution)	Ref
11	France	2010	46	Nil	Recurrent abscess with fistulas	*Actinomyces neuii*	Not specified	None mentioned	Recurrent abscesses	No improvement with antibiotics. Tumorectomy of the breast. No recurrence after 6 months.	22
12	Spain	2010	48	Nil	Painful erythematous fluctating left breast lump	*Actinomyces neuii*	Culture positive. Confirmed with 16S rRNA sequencing	None mentioned	1	Resolved with surgical debridement and a course of oral penicillin V	23
13	Switzerland	2011	67	Aortic valve replacement 2 months previous	3 day history of painful 12 cm swelling and hyperaemia of the left breast	*Actinomyces europaeus*	Culture positive. Confirmed with 16s rRNA and 16s rDNA sequencing	Mixed anaerobic flora	1	3 weeks of oral amoxicillin-clavulanic acid followed by 15 months amoxicillin. At 3 months 3 sinuses formed. Resolved at follow up 6 months later with scar formation.	24
14	India	2012	50	Nil	6 month history of intermittently discharging 6x4 cm right breast mass	*Actinomyces israelii*	Sulphur granules. Morphology on culture.	None mentioned	1	No information	25
15	India	2012	61	Diabetes	6 month history of 5 x 6 cm mass in left breast	Not isolated on culture	Histopathologic examination	None mentioned	1	Treated with unspecified antibiotics. Doing well on follow up.	26
16	India	2012	32	Nil	3 week history of 7 x 8 cm right breast mass	Not isolated on culture	Histopathologic examination	None mentioned	1	Resolved after a course of unspecified antibiotics.	27
17	USA	2013	40	Nil	2 week history of 3 cm tender right breast mass with overlying erythema	*Actinomyces odontolyticus*	Not specified	None mentioned	1	Cefalexin for 1 week with resolution after 2 weeks of oral penicillin V.	28

Table 4. Combined number and species of *Actinomyces* breast infections from tables 1-3 and from reference 7.

	Lothian cases*	Anaerobe Ref Unit cases†	Published cases‡	Total
A. europaeus	5	12	1	18
A. funkeii	0	2	0	2
A. israelii	0	0	4	4
A. massiliense	0	1	0	1
A. meyerii	0	0	4	4
A. neuii	3	13	3	19
A. odontolyticus	1	5	4	10
A. radingae	3	11	1	15
A. turicensis	0	15	1	16
A. urogenitalis	0	2	0	2
A. viscosus	0	0	1	1
A. species	1	0	4	5
Actinobaculum schaalii	1	0	0	1
Not cultured	0	0	4	4
Total	14	61	27	102
	* Two Lothian cases had two different *Actinomyces* sp. isolated	† Isolates referred from Lothian removed from this column to avoid double counting	‡ Includes 10 cases from ref 7 with no clinical details	