Necrotizing fasciitis

David V. Seal

Necrotizing fasciitis continues to occur due to beta-haemolytic streptococci but is now also recognized as being due to *Vibrio* spp. in fishermen and those in contact with warm water in the Gulf of Mexico and South-East Asia, including Hong Kong. Magnetic resonance image scanning has identified the extent of fasciitis and soft tissue oedema infiltrating fascial planes prior to necrosis presenting clinically and is a useful tool in early diagnosis. Surgical debridement or incisional drainage remains essential. An enhanced bactericidal response against beta-haemolytic streptococci has been found with a combination of penicillin and clindamycin. Intravenous immunoglobulin has been shown to reduce mortality if the necrotizing fasciitis is associated with the toxic shock syndrome, by decreasing the superantigen activity of the beta-haemolytic streptococci on cytokine release by T cells. Curr Opin Infect Dis 14:127–132.

Department of Diagnostic Medical Microbiology, St Mary's Hospital, Medical School, London, UK

Correspondence to David V. Seal, Department of Diagnostic Medical Microbiology, St. Mary's Hospital, Ground Floor, Medical School, Praed Street, London W2 1PG, UK
e-mail: eyehealth@compuserve.com

Current Opinion in Infectious Diseases 2001, 14:127–132

Abbreviations

BHS	beta-haemolytic streptococci
GABHS	group A beta-haemolytic streptococci
MRI	magnetic resonance imaging
TSS	toxic shock syndrome

0951-7375

Introduction

The term necrotizing fasciitis was first used to describe rapidly spreading gangrene of the skin and subcutaneous tissues above the fascial layer in the 1950s. At the time, pathogenesis was described due to the beta-haemolytic streptococci (BHS) group A (*Streptococcus pyogenes*), as a community-acquired infection and a synergistic infection of aerobes and anaerobes mostly acquired with gun shot injury or neoplasms of the gastrointestinal tract [1]. Necrotizing fasciitis is still the preferred term today. A new organism, *Vibrio vulnificus* (described below), is now recognised as causing a distinctive clinical syndrome of rapidly progressive necrotizing fasciitis, septicaemia and death while several other vibrios have also been incriminated.

New methods of diagnosis have been pursued with computerized tomography and magnetic resonance imaging (MRI) and an earlier diagnosis of impending necrosis can be made. Identification still depends on bacterial culture. New approaches to treatment include combination therapy with penicillin and clindamycin (for an enhanced bactericidal effect) and intravenous immunoglobulin (to reduce superantigen activity on T cells and cytokine production) with surgical excision, debridement or fasciotomy.

Necrotizing fasciitis due to beta-haemolytic streptococci

Necrotizing fasciitis due to beta-haemolytic streptococci is considered below for diagnosis and treatment.

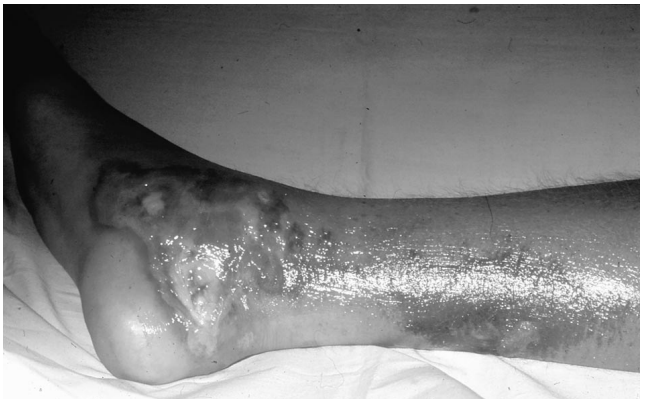
Clinical diagnosis

Fulminating necrotizing fasciitis is now recognised acutely due to Lancefield group A BHS (GABHS) (*S. pyogenes*) and in a sub-acute variety due to groups C and G [2]. It affects a wide age group from 15 to 90 years and especially those with diabetes, minor or major trauma or some degree of immuno-compromisation. Individual patient immunity to the M protein of the BHS cell wall (including the ability to opsonise the cells), previous exposure to the toxins and the numbers and site of the inoculated bacteria in the tissue all determine the outcome [3•]. The clinical disease is expressed by the organism spreading through tissue above the deep fascia causing thrombosis of vessels resulting in gangrene of subcutaneous fat and dermis [2]. This spreading thrombosis prevents penetration of antibiotics into the tissue allowing the suppurative process to progress often up an arm or a leg.

Clinical signs are distinguishable from erysipelas and cellulitis. The former have a well-defined border and may blister profusely, while the latter are associated with lymphangitis and blister only occasionally [3•]. Necrotizing fasciitis presents typically with patchy discoloration of the skin with pain and swelling but without a defined margin and lymphangitis. Within several days irregular dusky blue and black patches appear with bullae. As the infection spreads, frank gangrenous areas appear intermingled with bullae and patches of more normal skin (Fig. 1). The patient may develop bacteraemia and toxic shock syndrome (TSS) at an early stage, which often presents in a young previously healthy person [4]. There will be fever, prostration, hypotension, a psychotic effect with rejection of the illness and severe local pain at the site of the lesion, which can be remarkably small. Examination of the skin may be required at an early stage to detect subtle evidence of a soft tissue infection or small violaceous bullae. This situation can rapidly advance to multi-organ failure, acute respiratory distress syndrome, renal impairment, coagulopathy, liver abnormalities and generalised erythroderma [4].

In a retrospective study aggressive surgical management has been shown to reduce mortality from 38% to 4% [5]. Although retrospective studies are often biased [3], in this case with more severe disease having early surgery, this has also been the experience of other workers, albeit retrospectively [2]. The authors recommended wide excision and debridement, appropriate antibiotics and intensive systemic support if TSS is experienced. In an important community-based study of GABHS necrotizing fasciitis, an incidence was found of 0.4 per 100 000 with a median age of 58 years. 79% of these cases were community-acquired, 11% nosocomial and 10% acquired in a nursing home [6]. 47% were associated with TSS, 46% were bacteraemic and 34% died. Outcome was not correlated with the M type of the BHS nor its possession of 'spe' genes.

Figure 1. Acute necrotizing fasciitis due to GABHS



Reproduced with permission from [3•].

In contrast, less severe subacute infection can occur with BHS groups C and G in the elderly [2,3•]. Patients are not bacteraemic, the infection is relatively localised and responsive to early or late debridement of the eschar.

Computerised tomography and MRI have been used to assist in clinical diagnoses [7–9,10•]. In a study of 20 patients with necrotizing fasciitis to investigate the use of computerised tomography, asymmetric fascial thickening and fat stranding were seen in 16 cases, gas tracking along fascial planes in 11 cases and abscesses were found in seven. Computerised tomography appeared to be useful as an adjunctive diagnostic tool [6,11]. MRI has been used to define the extent of the fasciitis [7]. It has identified soft tissue oedema infiltrating the fascial planes of the entire chest wall prior to local gangrenous cutaneous signs of infection by many hours, allowing rapid surgical incisional drainage with success [8]. Confusion can occur with MRI, however, between cellulitis and necrotizing fasciitis [9]. Because the sensitivity of MRI exceeds its specificity, MRI can over-estimate the extent of deep fascial involvement. A negative result with no deep fascial involvement is useful to exclude necrotizing fasciitis [9] or to determine the depth of soft-tissue involvement [10•].

Microbiologic diagnosis

Blister fluid, discharge from open lesions, frank pus and any skin sepsis should be sampled for microscopy with Gram stain and cultured for BHS, aerobic and anaerobic bacteria. Blood cultures should also be collected [3•,6]. An early serum sample should be stored for later assay with samples collected after one, two and three weeks, for antibodies to streptolysin O, hyaluronidase and DNaseB. Samples of tissue removed at debridement should be ground up with antibiotic inhibitors and incubated in a fluid enrichment medium: this is much better for isolation than for swabs [3•]. A search should be made of the patient, family or contacts for the primary site of infection [6].

In cases of Fournier's gangrene, which is necrotizing fasciitis of the genitalia, cultures should be targeted at *Staphylococcus aureus* or *E. coli* aerobically and anaerobes such as clostridia, bacteroides or anaerobic streptococci [12].

Diagnosis of tissue infection with GABHS can also be made by polymerase chain reaction for detection of streptococcal pyrogenic exotoxin B (*speB*) gene. In a study of tissue biopsy specimens from patients with necrotizing fasciitis, an assay for *speB* was detected from

ten individuals with necrotizing fasciitis from whom GABHS were isolated, and was negative from 11 patients who were culture-negative or lacked serologic evidence of infection [13]. This polymerase chain reaction test may be useful for further investigating tissue from culture-negative necrotizing fasciitis patients but provides no additional information if the tissue is culture-positive.

Treatment

S. pyogenes remains fully sensitive to the bactericidal action of benzyl penicillin. Treatment should be given by the intravenous route and in large doses (at least 14.4 grams or 24 mega-units per day) as 4 mega-units every four hours. This will give an expected peak and trough of 40 to 80 and 1 to 2 mg/l respectively [14].

Clinical success is not always obtained. This can be due to a number of factors including disseminated intravascular coagulation. In addition, antibiotics will not penetrate into infected tissue in the presence of widespread thrombosis so that heparinization should be considered to aid antibiotic perfusion into tissue as well as to control any associated disseminated intravascular coagulation from *S. pyogenes* bacteraemia [3•]. Surgery is also indicated to debride thrombotic tissue expected to become necrotic, otherwise the spreading infected thrombotic process continues unabated.

There has been concern that high doses of benzyl penicillin IV may give a less effective bactericidal response than expected [15•]. In the laboratory this has been associated with persistent organisms in broth tube dilutions when a full killing effect was expected. Patients with deep invasive *S. pyogenes* infection were found to be more likely to have a favourable outcome if initial treatment included combination with a protein-synthesis inhibiting antibiotic, such as clindamycin in particular, compared with exclusive treatment with penicillins (83% versus 14%, $P = 0.006$) [15•]. Clindamycin has been found to be highly effective to treat *S. pyogenes* pharyngitis, to eradicate the streptococcal carrier state and to be highly effective in treatment of myositis in the mouse model test [15•]. Clindamycin is also known to decrease production of *S. pyogenes* toxins and enzymes even in subinhibitory concentrations [15•]. Clindamycin may reduce the release of toxins that act as superantigens leading to the production of cytokines, which are potential mediators of toxic shock syndrome and organ failure [15•,16]. Clindamycin should be administered in a dose of 900 mg every eight hours to give expected peak and trough levels of 10 to 12 and 1 to 2.5 mg/l respectively. Clindamycin should always be given for treatment of group G BHS infections since this particular BHS is known to show persistence both *in vitro* and *in vivo* in the presence of penicillin alone.

Adjunctive therapy can include the use of hyperbaric oxygen [17]. The use of hyperbaric oxygen is part of standard treatment for clostridial myonecrosis. Animal evidence supports its use in streptococcal myositis. Hyperbaric oxygen is also likely to be most effective if administered before end organ complications occur.

The rationale for the use of intravenous immunoglobulin

The ability of plasma (and intravenous immunoglobulin) to neutralise the proliferation and cytokine-inducing activity of superantigens, produced by GABHS causing necrotizing fasciitis and TSS, has recently been intensively studied. Supernatants prepared overnight from GABHS cultures produce superantigen activity and can be used for neutralisation studies in the laboratory. It was first found in 1997 that plasma collected from 12 patients with severe invasive GABHS treated with intravenous immunoglobulin, with pre and post samples, neutralised superantigen activity and stopped pyrogenic exotoxins (*spe A*, *B* and *C*) produced by their respective isolates more effectively [18]. Additionally, only the postintravenous immunoglobulin plasma samples blocked cytokine production elicited by purified *spe*s, supporting the use of intravenous immunoglobulins as adjunctive treatment of severe GABHS.

In 1999, three studies assessed the role of protective humoral immunity. Kaul *et al.* [19•] found that intravenous immunoglobulin enhanced the ability of patient plasma to reduce T cell production of interleukin-6 and tumour necrosis factor α in addition to bacterial mitogenicity. Basma *et al.* [20] compared 23 patients with severe GABHS with 12 non-severe cases and healthy control individuals. They found that levels of anti-M1 bactericidal antibodies and antistreptococcal superantigen neutralizing antibodies were significantly lower in both groups compared with control individuals ($P < 0.01$) but that there was no statistical difference between them. This suggests that other immunogenetic factors regulating the superantigen response may influence the severity of the clinical disease. Cawley *et al.* [21] studied the response of a patient with severe GABHS necrotizing fasciitis and TSS to intravenous immunoglobulin, in conjunction with surgical excision and antibiotics, and concluded that it may be a useful adjunctive treatment.

Mascini *et al.* [22] studied 53 patients with invasive GABHS. Absence of antibodies against *spe A* or *B* was a risk factor for developing invasive streptococcal disease. TSS and mortality were associated with a lack of anti-*spe A* antibodies. Patients infected with *spe A* producing GABHS developed anti-*spe A* antibodies in convalescent sera.

Necrotizing fasciitis due to *Vibrio* species

Vibrionaceae comprise three genera: *Vibrio*, *Aeromonas* and *Plesiomonas*. These comma-shaped Gram negative rods are all aquatic organisms associated with shell fish. All *Vibrio* spp. except *V. vulnificus* usually cause intense diarrhoea. A purulent wound infection has been reported with *V. cholerae* non-01 associated with a tropical fish tank [3•].

A. hydrophila is the predominant clinical pathogen mainly causing wound infection in the summer months associated with minor trauma and exposure to river or sea water. It can also cause septicaemia and necrotizing fasciitis, often in a diabetic or immunocompromised patient [23,24•].

Necrotizing fasciitis due to *Vibrio vulnificus*

V. vulnificus, first described in 1979, multiplies well in warm water ($>20^{\circ}C$) with a salt concentration of 0.7 to 1.6%. It is found in warm coastal waters such as the Gulf of Mexico, S. America, Asia (Thailand, Taiwan, Hong Kong) and Australia but is also reported in small numbers off Belgium and Scandinavia. It is found in oysters, crustaceans and fish, which may be the source of infection with or without trauma.

Necrotizing fasciitis due to *V. vulnificus* biotype 1 is severe [24•]. It occurs after minor trauma in fishermen and in those in contact with fish or sea water. Vibrio infections occur in the hot summer months from mid-May to mid-September in contrast to *S. pyogenes* infections, which tend to be concentrated in winter months [25].

Patients are often over 50 years-of-age and compromised with other conditions, particularly chronic liver dysfunction such as cirrhosis, or diabetes. The infection presents after one day with swelling, pain, tenderness, ecchymoses and blistering. Predominant skin lesions have been reported as oedema and subcutaneous bleeding, with ecchymosis and purpura, rather than superficial necrosis as is seen in *S. pyogenes* infection [25]. Systemically, there are fevers, rigors and hypotension. Septicaemia with *V. vulnificus* is a prominent feature, more so than with *S. pyogenes*. This leads to rapid multi-organ failure within 24 hours, which presents as encephalopathy (Glasgow coma score <6 off sedation), hepatic failure (bilirubin >120 μmol/l), renal failure (serum creatinine >350 μmol/l), respiratory failure ($FiO_2<150$) and most importantly disseminated intravascular coagulation (platelet count $<50\,000$/ml).

Patients with necrotizing fasciitis due to *V. vulnificus* progress more rapidly than *S. pyogenes* [25]. Early diagnosis and extensive debridement is essential to save life as otherwise this disease has an almost 100% mortality. While *Aeromonas* may infect muscle, this is unusual with *V. vulnificus* so that, in principle, debridement and not amputation is needed although this decision must be made uniquely for each case. Computerised tomography and magnetic resonance imaging are useful for locating the site and depth or extension of the infection as discussed above for GABHS. Cultures should be collected from blood, blister fluid, wound and stool specimens. Gram stains show curved bacilli with or without pleomorphic forms.

Antibiotics should be commenced in high dosage but are additional to surgical intervention. A combination of ceftazidime (or cefotaxime) with ciprofloxacin (or another quinolone) or tetracycline (or doxycycline) should be given in maximum dosage.

The first well-defined outbreak from a common source was described in Israel in 1996 [26••]. This was also the first recognition of this infection in countries bordering the Mediterranean Sea. It involved 62 cases of wound infection and bacteraemia in people who handled fresh whole fish (*Tilapia* spp.–St Peter's fish) cultivated in artificial fish ponds, purchased live and needing to be descaled. The isolates were of biotype three and had the same polymerase chain reaction–restriction fragment length polymorphism pattern. The incubation time varied from three hours to two days with a median of 12 hours. 57 patients developed cellulitis, four had necrotizing fasciitis and one developed osteomyelitis but there were no deaths. This contrasts with biotype 1 infections for which there is an expected mortality of 20% to 50%. Since ceasing to sell live fish, descaling and degutting the fish prior to marketing and selling the fish on ice, only three cases of invasive *V. vulnificus* infection have occurred in the last three years.

Necrotizing fasciitis due to other Vibrios

There are two other species currently recognised as being able to cause necrotizing fasciitis.

Vibrio parahaemolyticus

Septicaemia and acute necrotizing fasciitis involving the whole arm has been described in one patient from Singapore caused by *V. parahaemolyticus*. The 65-year-old patient had cut his arm while cleaning uncooked crabs. He presented in shock with erythema and swelling of the soft tissue of the right arm from the wrist to the axilla [27]. The patient was managed with intravenous fluid replacement, fasciotomy, antibiotics (ceftazidime and doxycycline) and intensive care for two days. Blood cultures yielded growth of the organism fully sensitive to antibiotics. The wounds healed gradually after two weeks. Patients with liver disease, alcoholism and diabetes are found to be more at risk of this infection. This is relevant to Singapore where 6% of the population

are hepatitis B carriers with some developing cirrhosis. The ambient sea temperatures in tropical areas such as Singapore (and the Gulf of Mexico) promote growth of Vibrios with sea water exposure and minor trauma being risk factors. These infections must be treated aggressively for successful recovery.

Photobacterium damsela (Vibrio damsela)

This isolate is similar to other species of *Vibrio* which are halophilic Gram-negative curved rods. Infection has been described due to this organism in a 43-year-old man following a laceration to the leg caused by a stingray fish in Tampa Bay, Florida [28]. After three days there was septicaemia and necrotizing fasciitis of the right tibial region. Deep surgical debridement of skin and fascia to the muscle layer was performed and intravenous antibiotics given (cefazolin, doxycycline and tobramycin). The organism was grown from wound cultures and was fully sensitive to antibiotics. The patient recovered needing split-skin grafts. *P. damsela* is a pathogen for both immunocompromised and healthy hosts and can cause rapid fulminant infection with death [28].

Conclusion

Necrotizing fasciitis remains an important cause of morbidity and mortality. In cold or temperate climates, community-acquired infection is usually due to the beta-haemolytic streptococci, especially *S. pyogenes*, while in summer months of hot climates there is an increasing recognition of this infection due to *Vibrio* spp. Both types are characterised by previous minor trauma but a salt water source is also involved with infection by Vibrio spp. The infection is more common in immunocompromised, diabetics or patients over 40 years old, especially those with some type of underlying disease such as cirrhosis. Management is similar for both types of infection, requiring acute surgical debridement, intravenous antibiotics, fluid replacement and intensive care. Mortality can vary from 5% to 50% depending on the length of incubation of the infection prior to presentation, its recognition by the clinical team and appropriate aggressive therapy.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1 Stevens DL. Streptococcal toxic shock syndrome associated with necrotizing fasciitis. Ann Rev Med 2000; 51:271–288.

2 Jarrett P, Rademaker M, Duffill M. The clinical spectrum of necrotising fasciitis. A review of 15 cases. Aust NZ J Med 1997; 27:29–34.

3 • Seal DV, Hay RJ, Middleton KR. Skin and Wound Infection – investigation and treatment in practice. London: Martin Dunitz; 2000.
A useful new book on clinical, microbiological and epidemiological aspects of skin and wound infection.

4 Drage LA. Life-threatening rashes: dermatologic signs of four infectious diseases. Mayo Clin Proc 1999; 74:68–72.

5 Bilton BD, Zibari GB, McMillan RW, *et al.* Aggressive surgical management of necrotizing fasciitis serves to decrease mortality: a retrospective study. Am Surg 1998; 64:397–400.

6 Kaul R, McGeer A, Low DE, *et al.* Population-based surveillance for group A streptococcal necrotizing fasciitis: clinical features, prognostic indicators and microbiologic analysis of seventy-seven cases. Am J Med 1997; 103:18–24.

7 Brothers TE, Tagge DU, Stutley JE, *et al.* Magnetic resonance imaging differentiates between necrotizing and non-necrotizing fasciitis of the lower extremity. J Am Coll Surg 1998; 187:416–421.

8 Drake DB, Woods JA, Bill TJ, *et al.* Magnetic resonance imaging in the early diagnosis of group A BHS necrotizing fasciitis: a case report. J Em Med 1997; 16:403–407.

9 Schmid MR, Kossmann T, Duewell S. Differentiation of necrotizing fasciitis and cellulitis using MR imaging. Am J Roentgenol 1998; 170:615–620.

10 • Revelon G, Rahmouni A, Jazaerli N, *et al.* Acute swelling of the limbs: magnetic resonance pictorial review of fascial and muscle signal changes. Eur J Radiol 1999; 30:11–21.
A useful presentation of the clinical relevance in decision-making of MRI.

11 Wysoki MG, Santora TA, Shah RM, Friedman AC. Necrotizing fasciitis: CT characteristics. Radiol 1997; 203:859–863.

12 Basoglu M, Gul O, Yildirgan I, *et al.* Fournier's gangrene: review of fifteen cases. Am Surg 1997; 63:1019–1021.

13 Louie L, Simor AE, Louie M, *et al.* Diagnosis of group A streptococcal necrotizing fasciitis by using PCR to amplify the streptococcal pyrogenic exotoxin B gene. J Clin Microbiol 1998; 36:1769–1771.

14 Stevens DL, Madaras-Kelly KJ, Richards DM. In vitro antimicrobial effects of various combinations of penicillin and clindamycin against four strains of *S. pyogenes*. Antimicrob Ag Chemother 1998; 42:1266–1268.

15 • Zimbelman J, Palmer A, Todd J. Improved outcome of clindamycin compared with beta-lactam antibiotic treatment for invasive *S. pyogenes* infection. Pediatr Infect Dis J 1999; 18:1096–1100.
A retrospective study of the value of treating with penicillin (or beta-lactam) and clindamycin as opposed to use of beta-lactams alone but with appropriate surgery in all patients. A prospective trial is warranted as retrospective studies can produce misleading results.

16 Sriskandan S, McKee A, Hall L, Cohen J. Comparative effects of clindamycin and ampicillin on superantigen activity of *S. pyogenes*. J Antimicrob Chemother 1997; 40:275–277.

17 Clark LA. Hyperbaric oxygen in the treatment of life-threatening soft-tissue infections. Resp Care Clin N Am 1999; 5:203–219.

18 Norrby-Teglund A, Low DE, McGeer A, *et al.* Superantigenic activity produced by group A streptococcal isolates is neutralized by plasma from IVIG-treated streptococcal toxic shock syndrome patients. Adv Exp Med Biol 1997; 418:563–566.

19 • Kaul R, McGeer A, Norrby-Teglund A, *et al.* Intravenous immunoglobulin therapy for streptococcal toxic shock syndrome – a comparative observational study. Clin Infect Dis 1999; 28:800–807.
A useful study reporting the benefits of treating streptococcal TSS with IVIG.

20 Basma H, Norrby-Teglund A, Guedez Y, *et al.* Risk factors in the pathogenesis of invasive group A streptococcal infections: role of protective humoral immunity. Infect Immun 1999; 67:1871–1877.

21 Cawley MJ, Briggs M, Haith LR, *et al.* Intravenous immunoglobulin as adjunctive treatment for streptococcal toxic shock syndrome associated with necrotizing fasciitis: case report and review. Pharmacotherapy 1999; 19:1094–1098.

22 Mascini EM, Jansze M, Schellekens JEP, *et al.* Invasive group A streptococci disease in The Netherlands: evidence for a protective role of anti-exotoxin A antibodies. J Infect Dis 2000; 181:631–638.

23 Lin CS, Cheng SH. *Aeromonas hydrophila* sepsis presenting as meningitis and necrotizing fasciitis in a man with alcoholic liver cirrhosis. J Formosan Med Assoc 1998; 97:498–502.

24 • Joynt GM, Gomersall CD, Lyon DJ. Severe necrotising fasciitis of the extremities caused by Vibrionaceae: experience of a Hong Kong tertiary referral hospital. Hong Kong Med J 1999; 5:63–68.
A useful paper on the clinical aspects of this infection.

25 Fujisawa N, Yamada H, Kohda H, *et al.* Necrotizing fasciitis caused by *Vibrio vulnificans* differs from that caused by streptococcal infection. J Infect 1998; 36:3113–3316.

26 •• Bisharat N, Agmon V, Finkelstein R, *et al*. Clinical, epidemiological, and microbiological features of *Vibrio vulnificus* biogroup 3 causing outbreaks of wound infection and bacteraemia in Israel. Lancet 1999; 354:1421–1424.
The first study of an epidemic of wound infection and necrotizing fasciitis in 62 cases due to a common source (farmed *Tilapia* spp. fish) from *V. vulnificus* biotype 3.

27 Lim TK, Stebbings AEL. Fulminant necrotizing fasciitis caused by *Vibrio parahaemolyticus*. Singapore Med J 1999; 40:596–597.

28 Barber GR, Swygert JS. Necrotizing fasciitis due to *Photobacterium damsela* in a man lashed by a stingray. N Engl J Med 2000; 342:824.