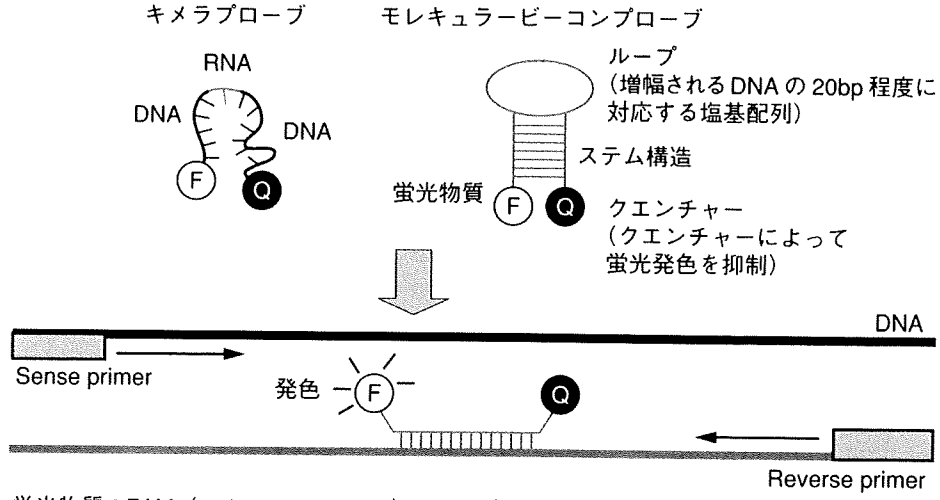

図 1　蛍光プローブを用いるリアルタイム PCR 法

DNA の増幅量と併行して蛍光発色量が増加するので，その発色量を 1 サイクルごとに自動的に測定する仕組みである。

従来の PCR 法では，40 サイクル以上の増幅を行うとどうしても非特異の DNA バンドが出現しやすいが，MB プローブは増幅された DNA に結合するため通常の PCR に比べて特異性がはるかに高い。陽性シグナルが早い段階で確認できれば，その時点で該当する微生物は陽性であると報告できる。

ちなみに，リアルタイム PCR 用の主な機器としては，Thermal Cyler Dice™（タカラバイオ），Mx-3000P™（アジテントテクノロジー），Light Cycler®（ロシュ・ダイアグノスティックス），リアルタイム PCR システム（アプライドバイオシステムズ・ジャパン）などがある。

まず，市中肺炎を念頭においてわれわれが最初に構築した呼吸器感染症起炎菌検出用キット（タカラバイオ）には，①肺炎球菌（自己融解酵素をコードする *lytA* 遺伝子），②インフルエンザ菌（16S rRNA 遺伝子），③マイコプラズマ・ニューモニエ（16S rRNA 遺伝子），④クラミドフィラ・ニューモニエ（16S rRNA 遺伝子），⑤レジオネラ・ニューモフィラ（16S rRNA 遺伝子と *mip*〔macrophage infectivity potentiator〕遺伝子），⑥β 溶血性レンサ球菌（16S rRNA 遺伝子と *slo* 遺伝子）用試薬が含まれている。レジオネラ・ニューモフィラと GAS 検索で二つの遺伝子を解析しているのは，それ以外の近縁の菌種とを区別するためであるが，この場合には一つのウエルで二つの遺伝子を解析するため，蛍光色素は FAM と ROX を用いている。

一方，検索できる呼吸系ウイルスは表 1 に示したように 13 種類である。すなわち，①Adenovirus（AdV），②Influenza virus A（Flu A），③Influenza virus B（Flu B），④Respiratory syncytial virus（RSV）subgroup A（RSV-A），⑤RSV subgroup B（RSV-B），⑥Parainfluenza virus1（PIV1），⑦Parainfluenza virus 2（PIV2），⑧Parainfluenza virus 3（PIV3），⑨Rhinovirus（RV），⑩Enterovirus（EV），⑪Coronavirus（CoV），⑫Human metapneumovirus（hMPV），⑬Human bocavirus（HBoV）である[8)~10)]。ただし，AdV と EV はタイプが多いため，それらすべてを検索できるようなプライマーは不可能で，主なタイプのみ検索可能である。

DNA/RNA 抽出から結果判定まで

検査材料処理から結果を得るまでのプロトコールを図 2 に示す。胸水，髄液，関節液などの DNA/RNA を直接抽出する検体と，上咽頭ぬぐい液，中耳検体，喀痰は 1 ml のブロスに混釈後その抽出操作を行う。われわれは，DNA/RNA の抽出には EXTRAGEN II®核酸抽出キット（東ソー）を用いている。12,000 rpm，3 分の簡単な遠心操作が 2 回入るが，所要時間は 10〜15 分程度である。最後に 40 μl の RNase free の蒸留水を加えて抽出

表 1　検索対象とした呼吸器系ウイルス

ウイルス（略称）	核酸の種類	増幅する目的遺伝子
①Adenovirus（AdV）	DNA	ヘキソン（hexon）
②Influenza virus A（FluA）	RNA	非構造タンパク（NS1）
③Influenza virus B（FluB）	RNA	核タンパク質（NP）
④RSV（subgroup A）	RNA	F タンパク（F）
⑤RSV（subgroup B）	RNA	F タンパク（F）
⑥Parainfluenza virus 1（PIV1）	RNA	ヘマグルチニン・ノイラミニダーゼ（HN）
⑦Parainfluenza virus 2（PIV2）	RNA	ヘマグルチニン・ノイラミニダーゼ（HN）
⑧Parainfluenza virus 3（PIV3）	RNA	ヘマグルチニン・ノイラミニダーゼ（HN）
⑨Rhinovirus（RV）	RNA	非翻訳領域（5'NCR）
⑩Enterovirus（EV）	RNA	非翻訳領域（5'NCR）
⑪Coronavirus（CoV）	RNA	スパイク糖タンパク（S）
⑫Human metapneumovirus（hMPV）	RNA	核蛋白質（NP）
⑬Human bocavirus（HBoV）	DNA	非構造タンパク（NP-1）

Adeno は 16/51 血清型を増幅する。
Entero は coxsackie A9，A16，B5，B6，echo 6，11，30，entero 71 を増幅する。

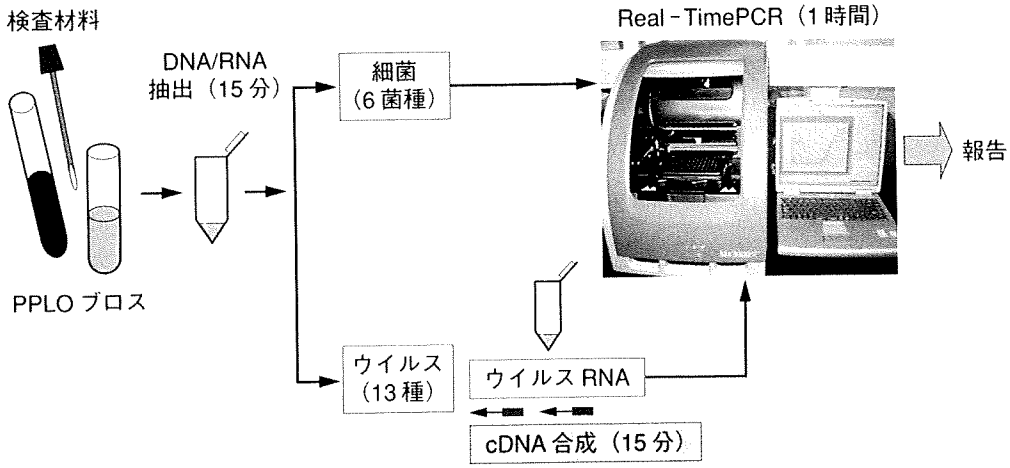

図 2　Real-time PCR による細菌/ウイルスの迅速検索プロトコール

液とする。

細菌検索用には，あらかじめ 6 菌種用 DNA 増幅試薬が分注された 8 連チューブに DNA 抽出液を 2μl ずつ加え，ただちにリアルタイム PCR を実行する。ウイルス検索には，まず cDNA 合成が必要であるが，この合成には 15 分で終了する PrimeScript™ RT reagent kit（タカラバイオ）を用いている。合成後には，細菌と同様にあらかじめ試薬が分注された 8 連チューブに cDNA サンプルを 2μl ずつ加え，リアルタイム PCR を実行する。

検体処理から結果が得られるまでの所要時間は 2 時間弱である。細菌やウイルス由来の DNA / RNA が多く含まれるサンプルでは，陽性反応を示す蛍光シグナルがサイクル数の早い段階で確認できるので，短時間で結果が判明する。

なお，DNA の増幅サイクル数は 40 サイクルまでとし，陽性反応が 35 サイクル以上のウイルスについては，電気泳動によって目的 DNA 断片か否か確認した方がよい。

リアルタイム PCR の感度と特異度

図 3 には，細菌 6 菌種の感度を示す。反応チューブあたりの遺伝子コピー数で 10^6〜10^1まで

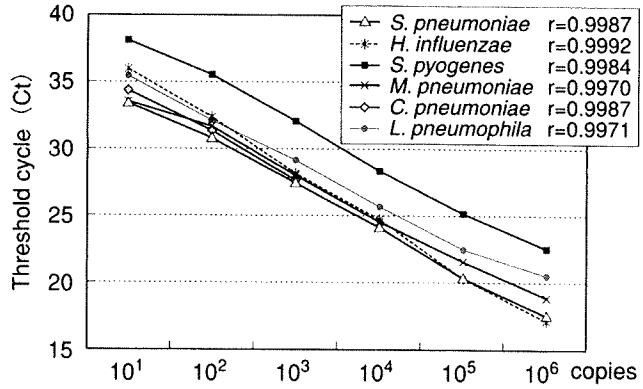

図 3　6 菌種の感度：Ct 値と目的遺伝子のコピー数の相関

反応チューブ当り 10^1コピーは検査材料当り 5×10^2コピーに相当。

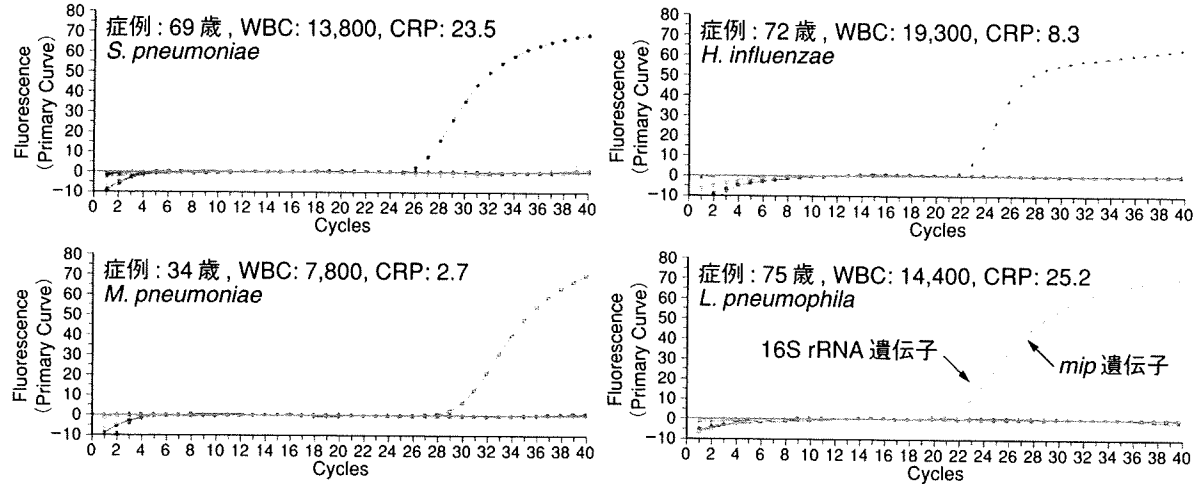

図 4　リアルタイム PCR による成人市中肺炎例の原因菌の迅速検査

喀痰から EXTRAGEN II キットを用いて DNA を抽出した後，cycleavePCR® 呼吸器系感染症起因菌検出キットを用いて，Thermal Cycler Dice™ Model TP800（タカラバイオ）でリアルタイム PCR を実行している。早い段階で陽性反応を示す程，該当する細菌量が多い。Legionella では *mip* 遺伝子と 16S rRNA 遺伝子の両方が陽性反応を示しているため，*L. pneumophila* と判定される。

示してあるが，チューブあたり数コピー存在すれば，32〜38 サイクルで陽性反応を示す感度となっている。ただし，細胞壁がやや強固で溶菌しがたいβ溶血性レンサ球菌では，5〜10 コピーで 38 サイクル陽性，肺炎球菌，インフルエンザ菌，マイコプラズマ，およびクラミジアでは 33〜36 サイクルで同じ感度を示す。

ウイルスについてもほぼ同様の感度であるが，それぞれのウイルスに対して設計されたプライマーとプローブの特異性は 13 種それぞれのクロス反応によって確認しており，いずれも偽陽性は認められず，特異性は高いと判断した。

成人肺炎例への応用

図 4 には，成人市中肺炎例から採取された喀痰に対し，リアルタイム PCR を行った中から細菌陽性例について，症例の年齢，WBC，CRP の成績とともに示す。これらの検索には，私どもの MB プローブをもとに構築された CycleavePCR® 呼吸器系感染症起因菌検出キット（タカラバイオ）が使用されているが，並行して培養も実施されており，PCR の結果は正しかったことが確認されている。

PCR の場合には感度が高いゆえに，喀痰のよう

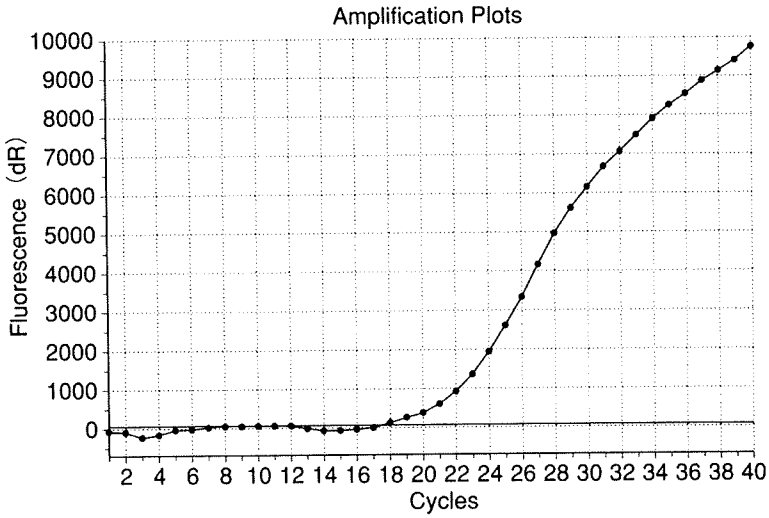

図 5　リアルタイム PCR による成人市中肺炎例のウイルス陽性例
図 4 で肺炎球菌が陽性（培養でも陽性）であった症例。本図にみられるように parainfluenza virus 3 も陽性であり，混合感染，あるいはウイルス感染が原発で肺炎球菌による続発感染を惹起したと推測される。使用機器は Mx-3000P™（アジレントテクノロジー）。

な無菌的でない検体で弱い陽性反応を示す菌が認められても，それが本当に原因かどうかは臨床検査値と併せて慎重に判断する必要がある。目的菌の中に黄色ブドウ球菌を加えていないのは，常在菌としてしばしば分離される本菌を原因菌と判断するのが極めて難しい理由からである。

図 5 には，図 4 の肺炎球菌陽性例で Parainfluenza virus 3 も陽性であった成績を示す。おそらく，ウイルスと細菌の混合感染，あるいはウイルス罹患後の肺炎球菌による続発感染と推定される。

図 6 には，外来受診から入院となった 188 例の成人肺炎例について，われわれが実施した細菌に対するリアルタイム PCR の成績を示す。残念ながらこの時点ではウイルスに対するリアルタイム PCR 法が完全には構築されていなかったので，細菌のみの成績となっているが，原因菌として最も多かったのは肺炎球菌，インフルエンザ菌，マイコプラズマであった。しかし，半数が起炎菌不明であった。

ここにはその成績は示さないが，小児に比べて原因不明が多い理由として，成人では①診療所を受診してから経過が思わしくなく病院を再受診している例が多いこと，したがって②大半の症例に対しすでに抗菌薬が投与されていること，③検査

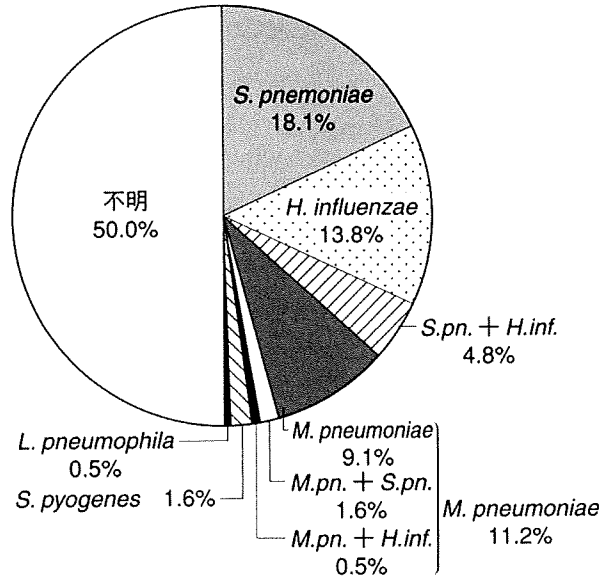

図 6　リアルタイム PCR による成人市中肺炎例の網羅的細菌検索（n＝188）

のタイミングを失していること，④検体が正しく採取されていないことが多い，などが挙げられる。

診断にはまず適切な検体が採取されていることが条件であり，それらが解決されれば，小児のように不明例は少なくなるであろう[11)〜13)]。

おわりに

急速に進行する耐性菌の増加に対し，抗菌薬の

適正使用が求められている。そのためには，本来原因微生物を迅速に確定し，それに基づいて抗菌薬が選択されることが基本である。また，臨床での感染症診断には，細菌のみならずウイルスをも網羅的に調べることも必要である。ウイルス単独感染であることが判明すれば，本来，対症療法のみで十分なはずである。

「正確な診断に基づいて治療抗菌薬を選択する」という視点に立つと，リアルタイムPCR法によるウイルスと細菌の網羅的検索法は，そのための有用な手段になり得るはずである。

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Streptococcus equisimilis 感染症

Invasive Streptococcus dysgalactiae subsp. *equisimilis infection in elderly patients*

特集

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変貌する感染症－人類の備えは十分か？

Key words 侵襲性感染症　β-溶血性レンサ球菌　*Streptococcus equisimilis*　高齢者

表題の *Streptococcus equisimilis* は，ヒトに対して感染症を惹起するレンサ球菌として，1996年に Vandamme ら[1]によって提唱された *Streptococcus dysgalactiae* subsp. *equisimilis* と呼ばれる新たな菌種名の略称である．

従来，ヒトから分離されるβ溶血性レンサ球菌の識別には，菌体表層に存在するC多糖体の違いを調べる抗血清による凝集反応，すなわち“Lacefield の分類”が広く用いられ，現在でも検査室レベルでは一般的に用いられている手法である．A群，B群，C群，G群，あるいはF群溶血性レンサ球菌と呼ばれることが多いが，これらは通称名であって正式な菌種名ではない．

そのなかで，ヒトにおける感染症との関わりでもっぱら重要視されてきたのは，A群溶血性レンサ球菌（*Streptococcus pyogenes*：GAS）とB群溶血性レンサ球菌（*Streptococcus agalactiae*：GBS）である．C群溶血性レンサ球菌（GCS）やG群溶血性レンサ球菌は病原性を有しない菌として扱われ，特殊病態下におけるヒトにおいてのみ，まれに重篤な感染症を引き起こす菌とみなされてきた．

しかし近年，わが国においては基礎疾患を有する高齢者人口の急速な増加に伴い，レンサ球菌感染症もまた変貌しつつある．ここでは，そのなかで臨床上最も罹患数が多く，注目され始めている *S. equisimilis* による侵襲性感染症について述べる．

S. equisimilis とは

表1には，ヒトに病原性を発揮するβ溶血性レンサ球菌について，Lacefield の抗血清による群別と，菌種を同定するうえでキーポイントとなるいくつかの性状を示す[2]．*S. equisimilis* の性状は *S. pyogenes* のそれときわめて似ているが，①溶血環とコロニーがGASよりも大きいこと，②アミノペプチダーゼ（PYR）試験が陰性であること，③β-D-グルクロニダーゼ活性は陽性であることが，重要な鑑別点である．

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0371-1900/08/¥50/頁/JCLS

表1　ヒトに病原性を示すβ溶血性レンサ球菌の主な性状
Manual of Clinical Microbiology（8版）のレンサ球菌の項を要約

菌名	Lancefield	コロニー	宿主	PYR(PyrA)	VP	β-GUR	β-GAR
S. pyogenes	A	大	ヒト　動物	＋	－	－	(＋)
S. dysgalactiae subsp. *equisimilis*	A, C, G	大	ヒト　動物	－	－	＋	－
S. anginosus group	A, C, G, F, Nontypable	小	ヒト　動物	－	＋	－	－
S. agalactiae	B	大	ヒト　動物	－	＋	(＋)	－

PYR：ピロリドニルアシルアミダーゼ(アミノペプチダーゼ)　β-GUR：β-D-グルクロニダーゼ
β-GAR：β-ガラクトシダーゼ　＋，－：90％以上　(＋)：50％前後

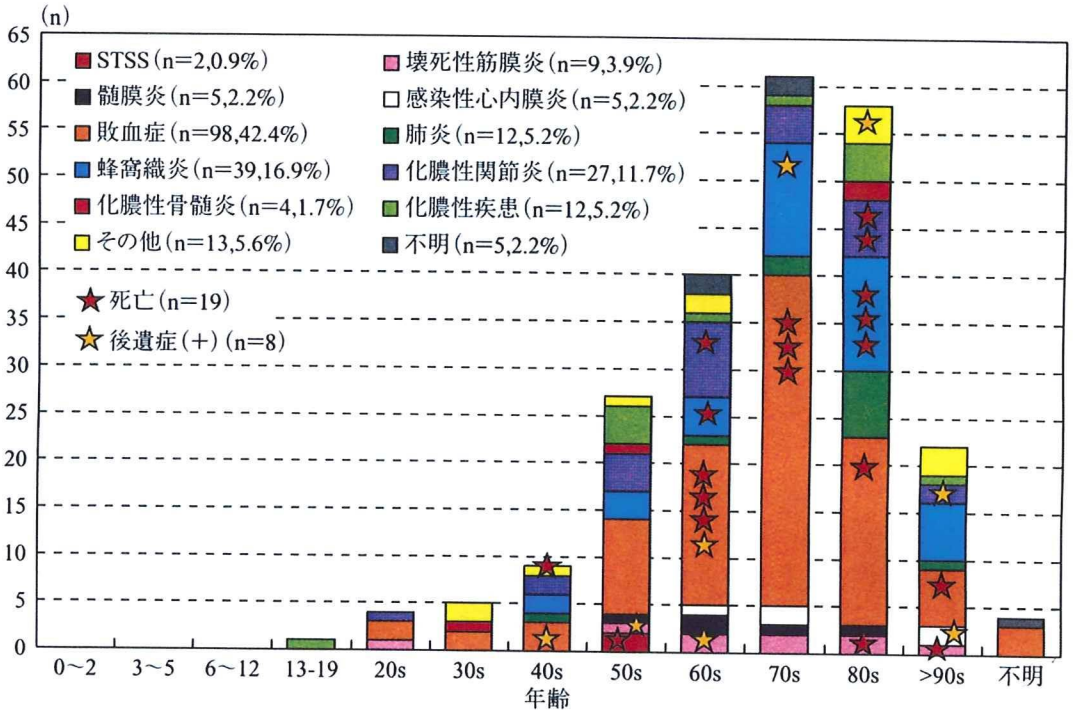

図1　*S. equisimilis* による侵襲性感染症名と症例の年齢分布，およびその予後(n=231)

S. equisimilis は，検査室で従来から実施されている群別ではG群に凝集することが最も多く，次いでC群，まれにA群に凝集する場合もある．つまり凝集試験のみでは正確な菌種名を付けることができない．現在，*S. equisimilis* のゲノム解析が進行中であるが，この新たな菌種はGASときわめて高い相同性を有していることが明らかになりつつあり，後述する病原性に関わる多くの遺伝子が両者に共通していることが注目されている．

S. equisimilis による侵襲性感染症の特徴

図1は，2006～2007年にかけて，第19回日本臨床微生物学会で企画されたワークショップ「侵襲性感染症とその検査に関する精度の検証」[3]において，全国186医療機関の検査室を通じて収集された231株の *S. equisimilis* 分離症例における年齢分布と疾患名，およびその予後を示したものである．

菌は血液からの分離例がほとんどであるが，その他に関節液，組織，閉鎖性膿汁からも分離されている．疾患としては敗血症(42.2％)が最も多く，次いで蜂窩織炎(16.9％)や化膿性関節炎(11.7％)が多い．例数は少ないが，壊死性筋膜炎(3.9％)，STSS(0.9％)，化膿性髄膜炎と感染性心内膜炎がそれぞれ2.2％ずつ認められる．*S. equisimilis* によって惹起される疾患をみると，GASによる疾

表2 *S. pyogenes* と *S. equisimilis* に見い出される主な病原遺伝子とコードされたタンパク機能

病原遺伝子	タンパク	機能
mrp (*fcrA*)	IgG 結合タンパク	IgG に結合し，貪食抵抗性を示す.
emm	M タンパク	抗オプソニン活性，菌体の付着，侵入に関与.
enn	IgA 結合タンパク	IgA に結合．貪食抵抗性は示されていない.
fba	フィブロネクチン結合タンパク	フィブロネクチンに結合すると同時に，H 因子とも結合し，抗オプソニン活性を示す.
sof	血清混濁物質 (オパシティファクター)	アポプロテアーゼとして働く．また，ヒトの細胞外マトリックス(ECM)と結合し相互作用する.
sclA	コラーゲン様タンパク	ヒト血清 LDL を吸収し，宿主細胞の$\alpha 2,\beta 1$インテグリンに結合．細胞侵入を助長する.
scpA	C5a ペプチダーゼ	補体成分 C5a を分解し，好中球などの遊走を阻害する.
sic	補体阻害物質(*emm*1型のみ)	補体成分と結合し，膜傷害性複合体(MAC)形成を阻害．また，lysozyme や difencin などの活性も阻害する.
speB	システインプロテアーゼ	Dick 毒素の 1 種．抗体の排除に関与.
slo	ストレプトリジン O	赤血球のコレステロールに結合し，溶血活性を示す．細胞傷害作用.
sagA	ストレプトリジン S	リン脂質に作用し，さまざまな細胞に対し傷害作用を示す.
ska	ストレプトキナーゼ	プラスミノーゲンアクティベーター．結果的にフィブリンを分解し，菌の拡散を助長.
hylP	ヒアルロニダーゼ	ヒアルロン酸を分解し，菌の組織への拡散を助長する.
sda	ストレプトドルナーゼ	DNA 分解酵素．病原性における役割は不明な点が多い.

患とほぼ同じであることが判るが，その原因は恐らく菌の病原性因子が共通していることに起因しているのであろう.

一方，発症年齢は図1からも明らかなように，20歳未満での発症例はきわめてまれで，50代から急速に症例数が増加し，70～80代での発症例の多いことが特徴である．この年齢分布は基礎疾患を有する年齢分布にオーバーラップするように思うのである．そして，発症例における基礎疾患の有無を調べると，全体の75％が実際に糖尿病や脳血管障害，肝・腎系の疾患，心疾患などを高率に抱えていた.

菌送付時に発症例の予後が記載されていた156例中，死亡例が19例(12.2％)，後遺症残存例が8例(5.1％)認められている．この率はGAS よりもやや低く，GBS よりも高い．また，これらの予後不良例は，入院後数日で不幸な転帰をとっているが，蜂窩織炎・蜂巣炎を含むSTSS や壊死性筋膜炎例ではもちろんのこと，敗血症例での死亡例も多い.

発症例の初期受診科を調べると，高齢者が多いためかGAS に較べて内科系診療科の受診例が約45％を占めていたが，時間外受診(救急)例も18.2％認められたこと，整形外科受診例が13.9％と予想以上に多いことが注目され，本菌では化膿性疾患の多いことが示唆される.

病原性に関わる因子

β溶血性レンサ球菌の病原因子については，1940年代から多くの先駆者によって病原性に関わる産生物の単離やその性状，あるいは抗体獲得機構が主なテーマとして扱われ，膨大な研究が行われてきている[4].

GAS とGBS についてはすでに全ゲノム解析が終了しており，*S. equisimilis* のそれも私達によってほぼ終了しているが，それらを比較することによってGAS と *S. equisimilis* はきわめて近縁の菌種であることが明らかになりつつある．このことは，GAS の染色体上に見い出される病原性遺伝子の多くが，*S. equisimilis* にも共通して見い出されるということを意味している.

表2 は，GAS と *S. equisimilis* に見い出される主な遺伝子とコードされたタンパク(酵素)，その

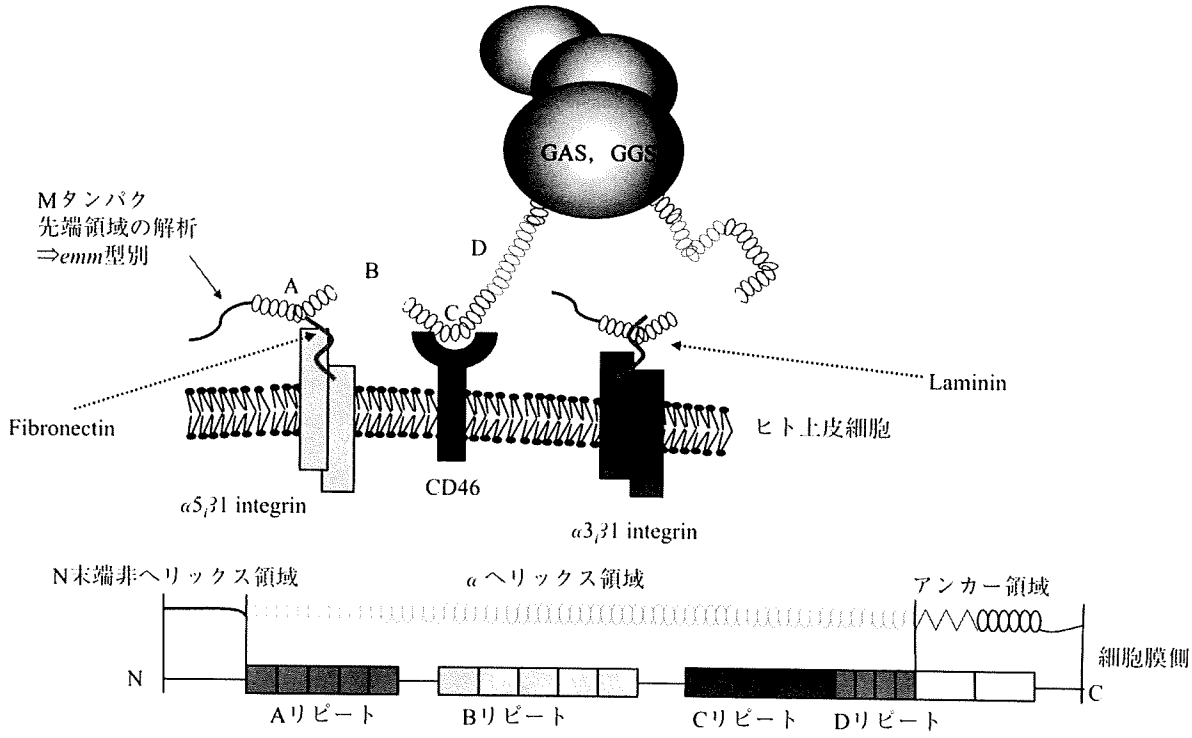

図2 *S. equisimilis* と *S. pyogenes* の菌体表層に存在するMタンパクの構造とヒト上皮細胞への付着機構
(Fischetti VA：Clin. Microbiol. Rev 2：285-300, 1989.
Rezcallah MS：Cell. Microbiol 7：645-653, 2005.
Wang B：GRAM-POSITIVE PATHOGEN 2nd Edition, 29-36, 2006. より一部改変)

機能についてまとめたものである．菌の病原性因子は，①宿主細胞への付着と侵入に関わるもの，②宿主免疫系からの回避，③そして侵入後における組織への拡散や傷害作用と，大きく3つに分けることができる．①に区分されるMタンパクは**図2**に示したが，②の機能も有している．その他には，IgG結合タンパク，IgA結合タンパク，フィブロネクチン結合タンパク，コラーゲン様タンパクなどが存在する．②には，Mタンパク，IgG，IgA両結合タンパク，フィブロネクチン結合タンパクの他に，補体成分C5aを不活化するペプチダーゼ，補体の膜傷害性複合体(MAC)形成インヒビターなどがあげられる．一方，③には組織壊死に関わるとされるストレプトリジンOやストレプトリジンS，組織間への菌の拡散を助長するストレプトキナーゼと，ヒアルロニダーゼ，システィンプロテアーゼなどがある．

診断上問題となるのは，本菌がストレプトリジンOを保持していることである．つまり，*S. equisimilis* 感染では感染後にASO価が上昇するので，菌が分離できなければどの菌種の感染であったのか区別できないのである．

Mタンパクをコードする *emm* 遺伝子による分子疫学

図2には，*S. equisimilis* の菌体表層に存在するMタンパクとその構造を示す．細胞膜から繊維状に伸びたMタンパクは，A～Dまでのリピート構造を呈しているが，先端のN末側の非ヘリックス領域が可変性に富んでいることから，この領域をコードする遺伝子解析を行うことによって疫学解析が可能である．菌ごとの解析データをCDCレファレンスセンターへ送信すると，*emm* 型が記されたmailが返信されてくる仕組みであるが，*S. equisimilis* の場合は *stG* あるいは *stC* 番号が付けられる．ちなみに，GASでは *emm* 番号が付けられる．

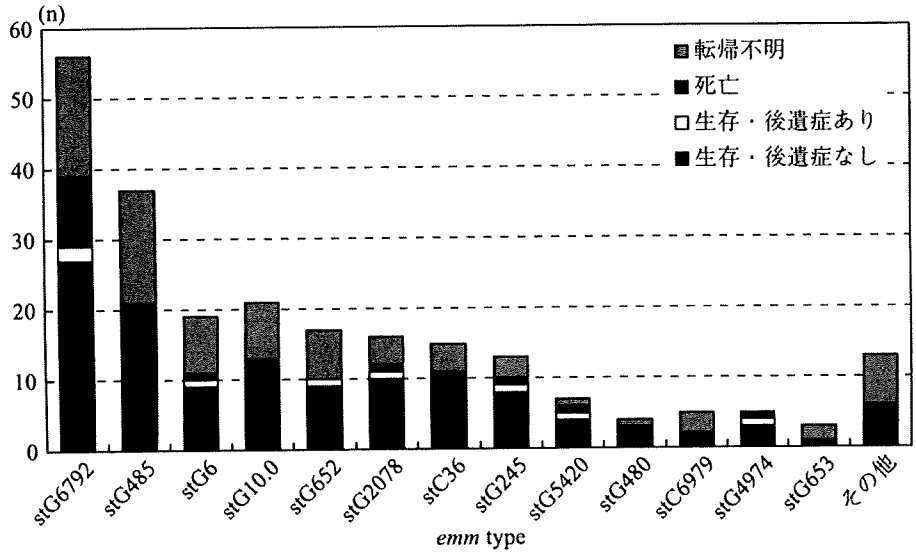

図3 *S. equisimilis* の *emm* 型と症例の予後との関係(n=231)

図3には侵襲性感染症由来の *S. equisimilis* における型別成績を示す．*stG*6792型が最も多く24.2%を占めている．しかも，この型の菌の分離例では，"死亡+予後不良例"が有意に多い．ちなみに，*stG*6792型菌は敗血症，蜂窩織炎，化膿性関節炎，感染性心内膜炎由来株に多く，膿瘍由来株には少ないことが特徴である．その他の型でも予後不良例が散見されるが，それらの症例では宿主側のリスクファクターの関与が大きいと思われる．

表3 *S. equisimilis* のβ-ラクタム系薬と Vancomycin 感受性(n=231)

抗菌薬	MIC range	MIC_{50}	MIC_{90}
経口薬			
penicillinG	0.008-0.016	0.016	0.016
ampicillin	0.016-0.031	0.031	0.031
amoxicillin	0.016-0.031	0.031	0.031
cefdinir	0.008-0.031	0.016	0.016
cefditoren	0.008-0.016	0.016	0.016
注射薬			
cefazolin	0.063-0.25	0.125	0.125
cefotiam	0.063-0.125	0.063	0.125
cefotaxime	0.008-0.031	0.016	0.016
panipenem	0.004-0.016	0.008	0.008
meropenem	0.008-0.016	0.016	0.016
vancomycin	0.25-1	0.5	0.5

治療抗菌薬

S. equisimilis に対する各種抗菌薬感受性は表3に示す．基本的には，本菌に対するβ-ラクタム系薬の感受性はGASのそれとほぼ同等で，耐性菌は認められていない．しかし，CEZ，CTMなどのセフェム系注射薬の抗菌力と殺菌力は，ペニシリン系薬やカルバペネム系薬に比して明らかに劣っている．一般的にはカルバペネム系薬にCLDMの併用，あるいはペニシリン系薬の大量投与にCLDMの併用が行われている．ちなみに，CLDMの併用は菌の毒素産生性を低下させるとする報告や，劇症型感染の早期であればヒト免疫グロブリン製剤の大量投与が有効であるとする報告もみられる．ただし，重症感染症例においては宿主側因子や受診のタイミングが予後に最も影響し，どのような補助療法がタイムリーになされるかも重要である．

侵襲性感染症の治療とは直接関係はないが，マクロライド系薬の耐性率は約10%，ニューキノロン系薬の耐性もわずかながら認められている．これらの株はすでに耐性遺伝子を保持しており，今後の耐性化動向には注意が必要であろう．

まとめ

病原性に乏しいとされてきた *S. equisimilis* がGASに匹敵する病原性を獲得してきた経緯について，Kaliaら[5]は両菌種の遺伝子を詳細に解析し，β溶血性レンサ球菌の進化の過程を推定している．はるか遠い昔にレンサ球菌は共通の祖先から枝分かれしたが，進化の過程においてGASの染色体DNAが大きな断片ごとGCSやGGSへ伝達されたとするものである．その後，染色体DNAの欠失や組換えが生じ，現在の *S. equisimilis* に至っているとする説である．

このような遺伝子伝達は，菌が保持する溶原化ファージによって生じるが，ファージによる遺伝子伝達は肺炎球菌や黄色ブドウ球菌など，グラム陽性球菌で古くから知られた現象でもある．

今後，基礎疾患を保持するヒトがなぜ急速に重篤化しやすいのか，臨床と基礎の両面からさらなる研究が必要であろう．

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ACKNOWLEDGMENTS

We thank Grant Crittenden for the English correction.

Conflict of Interest: The editor in chief has reviewed the conflict of interest checklist provided by the authors and has determined that the authors have no financial or any other kind of personal conflicts with this paper.

Author Contributions: Mutsuo Yamaya: taking care of the patient and preparation of the manuscript. Motoki Yoshida, Miyako Yamasaki, Katsutoshi Furukawa, and Hiroyuki Arai: taking care of the patient. Hiroshi Kubo: taking care of the patient and preparation of the figure.

Sponsor's Role: No sponsor support on this study.

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CO-INFECTION WITH RESPIRATORY SYNCYTIAL VIRUS SUBGROUP A AND *STREPTOCOCCUS PNEUMONIAE* DETECTED BY A COMPREHENSIVE REAL-TIME POLYMERASE CHAIN REACTION ASSAY IN AN ELDERLY PATIENT WITH COMMUNITY-ACQUIRED PNEUMONIA

To the Editor: Respiratory viruses, including community-acquired pneumonia (CAP) and bronchiolitis, account for a substantial portion of illnesses in elderly people.[1] Respiratory syncytial viruses (RSVs) and influenza virus are the most commonly identified viral pathogens in elderly subjects with acute respiratory diseases.[2] Respiratory syncytial viral pneumonia should be suspected during winter in patients with coryza, wheezing, low-grade fever, and patchy infiltrates whenever rapid testing does not detect influenza. An outbreak of RSV infection in a long-term care facility was recently documented using reverse transcription polymerase chain reaction (RT-PCR).[3] Here rapid diagnosis of co-infection with RS virus subgroup A and *Streptococcus pneumoniae* is reported in an elderly patient with CAP using comprehensive testing for pathogens using real-time PCR.

An 89-year-old man with diabetes mellitus and hypertension was hospitalized in January 2009 because of fever (38.6°C), cough, and respiratory distress for 2 days. Crackling rales were apparent bilaterally in the periscapular region. Gram stains of an endotracheal aspirate obtained at admission showed no evidence of respiratory pathogens such as *S. pneumoniae*. Laboratory findings included high blood urea nitrogen (BUN; 48 mg/dL, normal 10–15 mg/dL), creatinine (Cr; 1.58 mg/dL, normal 0.5–1.2 mg/dL), and C-reactive protein (CRP; 25.25 mg/dL (<0.3 mg/dL) and a normal white cell count (5,600/mm^3). Other laboratory data except for blood glucose (177 mg/dL) were within the normal range. A chest roentgenogram showed bilateral infiltrates in the mid-lung fields. Bilateral diffuse, patchy infiltrates adjoining bronchovascular bundles were confirmed according to computed tomography of the chest.

Real-time PCR to identify *S. pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Legionella pneumophila*, *Chlamydophila pneumoniae*, and *S. pyogenes*, as well as real-time RT-PCR to detect RSV A and B; influenza virus A and B; parainfluenza virus 1, 2, and 3; rhinovirus; enterovirus; human metapneumovirus; human bocavirus; and adenovirus were immediately performed on an aspirate obtained at admission, as previously described.[4–6] RSV subgroup A–specific primers used for real-time PCR included a sense primer (5′-AACAGATGTAAGCAGCTCCGTTATC-3′) and a reverse primer (5′-CGATTTTTATTGGATGCTGTACATTT-3′); a specific molecular beacon (MB) probe was 6-carboxyfluorescein-GCTGCCTGCCATAGCATGACACAATGGCTCCTGGCAGC-black hole quencher 1 (90 bp). *S. pneumoniae*–specific primers for real-time PCR included a sense primer (5′-CAACCGTACAGAATGAAGCGG-3′) and a reverse primer (5′-TTATTCGTGCAATACTCGTGCG-3′). A specific MB probe was 6-carboxyfluorescein-CGCGATCAGGTCTCAGCATTCCAACCGCCGATCGCG-black hole quencher 1 (319 bp). For RSV subgroup A, the F gene sequence (GenBank accession no. AF067125) was used; for *S. pneumoniae*, the *lytA* gene sequence (GenBank accession no. M13812), which codes for an autolysin enzyme, was used. Deoxyribonucleic acid and ribonucleic acid extraction was completed within 10 minutes, and real-time PCR was completed within 1.5 hours. Total time from sample processing to definitive results was no more than 3.0 hours. Based on known sensitivities for significant pathogens exhibited by real-time PCR, threshold cycle values of 33 or less were defined as positive.[4–6] Nucleic acids of RSV subgroup A (Figure 1, upper panel) and *S. pneumoniae* (Figure 1, lower panel) were clearly evident, with no indication of other respiratory bacterial or viral nucleic acids.

The patient was diagnosed with community-acquired co-infection by RSV subgroup A and *S. pneumoniae* and was treated intravenously with sulbactam-ampicillin (1.5 g twice daily for 6 days) and hydrocortisone (50–100 mg/d for 5 days). Oxygen (3 L/min) was administered through a nasal canula, and hydration was maintained. Fever and respiratory distress gradually abated. Laboratory abnormalities involving BUN, Cr, and CRP normalized; infiltrates decreased radiographically. Culture of the aspirate detected *S. pneumoniae*. The serotype was 11A; the minimum inhibitory concentration of penicillin G was 0.06 μg/mL, suggesting susceptibility. On hospital Day 3, a urinary antigen test detecting *S. pneumoniae* was positive. RSV infection was confirmed when anti-RSV antibody testing using complement fixation titer increased from less than 1:4 on admission to 1:512 over 1 month. After discharge, the patient had no symptoms such as cough or fever. No abnormalities

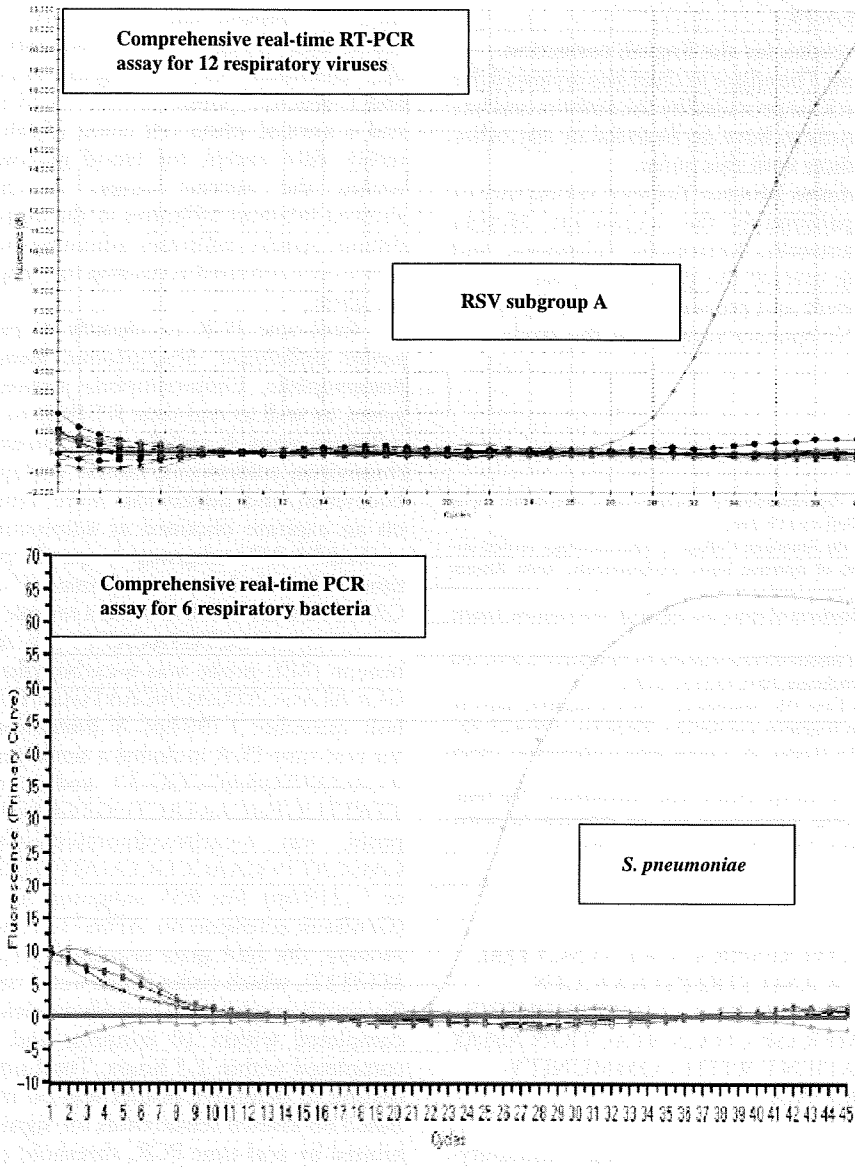

Figure 1. Target genes of respiratory syncytial virus (RSV) subgroup A (the fusion protein gene, above) and of *Streptococcus pneumoniae* (the *lytA* gene, below) were amplified using real-time polymerase chain reaction (PCR) performed upon an endotracheal aspirate in an elderly patient with community-acquired pneumonia. There was no indication of other respiratory bacterial or viral nucleic acids. RT = reverse transcription.

were demonstrable in a chest roentgenogram obtained at an outpatient clinic.

A panel to detect 20 human respiratory viruses was recently developed using multiplex PCR and a fluid microbead-based assay.[7] This method can detect influenza A virus subtypes H1, H3, and H5 but requires 5.0 hours for completion and cannot detect significant respiratory bacteria such as *S. pneumoniae*. Concurrent bacterial infection in elderly patients hospitalized for viral infection may cause acute respiratory distress and worsen prognosis. Influenza and RSV infections increase hospitalization rates, antibiotic use, and deaths in elderly nursing home residents each winter; undetected bacterial co-infection could be contributory.[8] A real-time PCR array, which can detect six bacterial pathogens including *S. pneumoniae* and 12 viruses, can rapidly identify causative agents of CAP in clinical settings.

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ACKNOWLEDGMENTS

Conflict of Interest: The editor in chief has reviewed the conflict of interest checklist provided by the authors and has determined that the authors have no financial or any other kind of personal conflicts with this paper.

This study was funded in part by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (19591187 to Dr. T. Takahashi).

Author Contributions: Takashi Takahashi was responsible for the study concept and design, interpretation of data, and preparation of the manuscript. Miyuki Morozumi, Naoko Chiba, Ryoko Asami, Kozue Kishii, and Somay Y. Murayama were involved in sample collection and treatment and in the comprehensive real-time PCR assay. Kimiko Ubukata was responsible for the study concept and design, analysis of data, and preparation of the manuscript.

Sponsor's Role: None.

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A CASE REPORT OF CRUSTED SCABIES WITH METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* BACTEREMIA

To the Editor: Ms. MC was an 85-year-old woman who was transferred from an outside hospital to a contact isolation bed on the geriatric inpatient service at our acute care hospital in New York City, NY. She originally presented with several days of increased lethargy, bloody discharge from her gastrostomy site, and a progressive non-pruritic skin rash.

She had been residing at a long-term care facility for the previous 5 years. She had a history of severe dementia and had been largely nonverbal, was bed-bound with limb contractures, and required feeding through gastrostomy.

Approximately 9 months before presentation, Ms. MC developed a scaly, eczematous rash on her upper back and hands. She saw a dermatologist and was treated with a topical corticosteroid. The rash did not improve and instead spread to the neck, ears, and palms of her hands.

Ms. MC saw a surgical consultant at the outside hospital, who performed a biopsy of the skin lesion. She was also found to have methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia. A transthoracic echocardiogram was performed that, although technically limited, did not find any obvious valvular vegetations. She was treated empirically for MRSA endocarditis with intravenous vancomycin.

Upon request by her family, the patient was then transferred to our acute care hospital in New York. She received a dermatology consultation and was found on skin scrapings to have scabies infestation. At this time, the biopsy results from the other hospital also revealed scabies mites. She was treated with oral ivermectin and topical permethrin, the latter poorly tolerated because of intense pruritus. Figure 1 shows the patient's skin findings 2 days after treatment with permethrin.

Despite continued treatment, her clinical course rapidly deteriorated, with the development of gram-negative rod sepsis and multi-organ system failure related to aspiration pneumonia. Fluid resuscitation and additional antimicrobial coverage failed to improve her condition. She received intravenous morphine and glycopyrrolate for symptom management as the goals of care were shifted to palliation. She died comfortably on hospital day 8.

Scabies is a common parasitic disease caused by the mite *Sarcoptes scabiei* that infects approximately 300 million people worldwide yearly.[1] Severe infections are especially common in elderly people residing in nursing homes, especially in patients who are immunocompromised, malnourished, and cognitively impaired.

This case demonstrates the importance of recognizing atypical presentations of scabies, especially crusted scabies, also known as Norwegian scabies, which has been associated with numerous outbreaks in nursing facilities.[2] It has a high infectivity rate because of its large parasite load; in classic scabies, only five to 15 mites live on the host,

ORIGINAL ARTICLE 10.1111/j.1469-0691.2009.03047.x

Clinical aspects of invasive infections with *Streptococcus dysgalactiae* ssp. *equisimilis* in Japan: differences with respect to *Streptococcus pyogenes* and *Streptococcus agalactiae* infections

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Abstract

Streptococcus dysgalactiae ssp. *equisimilis* (SDSE) is increasingly being identified as a pathogen responsible for invasive and non-invasive infections. We compared the clinical features of invasive SDSE infections with those of invasive infections caused by *Streptococcus pyogenes* (group A streptococcus (GAS)) and *Streptococcus agalactiae* (group B streptococcus (GBS)). Active surveillance for invasive SDSE, GAS and GBS was maintained over 1 year at 142 medical institutions throughout Japan. Clinical information was collected together with isolates, which were characterized microbiologically. Two hundred and thirty-one invasive SDSE infections were identified, 97 other patients had infections with GAS, and 151 had infections with GBS. The median age of the SDSE patients was 75 years; 51% were male and 79% had underlying diseases. Forty-two SDSE patients (19%) presented to the emergency department. Among the 150 patients (65%) for whom follow-up was completed, 19 (12%) died and eight (5%) had post-infective sequelae (poor outcome). Insufficient white blood cell responses (<5000 cells/μL) and thrombocytopenia on admission each suggested significantly higher risk of poor outcome (ORs 3.6 and 4.5, respectively). Of 229 isolates, 55 (24%) showed an stG6792 *emm* type, which was significantly associated with poor outcome (OR 2.4). Clinical manifestations of invasive SDSE infections were distinct from those of invasive GBS infections. Primary-care doctors should consider invasive SDSE infections when treating elderly patients.

Keywords: Invasive infections, non-invasive infections, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus dysgalactiae* ssp. *equisimilis*

Original Submission: 7 June 2009; **Revised Submission:** 23 August 2009; **Accepted:** 24 August 2009

Editor F. Allerberger

Clin Microbiol Infect

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Introduction

Invasive infections caused by β-haemolytic streptococci apart from Lancefield groups A and B, as well as by *Streptococcus pyogenes* (group A streptococcus (GAS) and *Streptococcus agalactiae* (group B streptococcus (GBS)), are reported increasingly worldwide [1,2]. The other streptococci include groups C, G, F and L; group G is notable because these streptococci can cause bacteraemia [3,4]. According to previous investigations [5], this group includes *Streptococcus dysgalactiae* ssp. *equisimilis* (SDSE), the *Streptococcus anginosus* group, and *Streptococcus canis*.

Recently, SDSE isolates possessing group G antigen have been recovered increasingly from severe invasive streptococcal infections [6]. Brandt *et al.* [7] characterized blood culture isolates of SDSE possessing Lancefield group A antigen. Infection with this pathogen (11 strains) was also sometimes found to lead to streptococcal toxic shock syndrome [8]. We have just completed whole genome analyses of two original isolates (GGS_124 (GenBank accession number

AP010935) and RE378) of SDSE, demonstrating a rate of genome overlap between this subspecies and GAS of 61–63%, whereas the overlap between the subspecies and GBS genomes was 15% (T. Akiyama, K. Ubukata & T. Kirikae, unpublished data).

However, active nationwide surveillance with a collection of large numbers of strains remains to be instituted, as for many years SDSE was considered to be non-pathogenic. We therefore collected isolates of this microorganism as well as of GAS and GBS, with accompanying detailed clinical information, from 142 medical institutions. Our aim was to compare clinical aspects of invasive diseases caused by SDSE with those caused by GAS or GBS in Japan.

Materials and Methods

Surveillance

Active laboratory-based surveillance for invasive SDSE, GAS or GBS infections was organized by the Laboratory of Molecular Epidemiology for Infectious Agents at the Graduate School of Infection Control Sciences, Kitasoto, Japan. SDSE included some isolates of the Lancefield C or A groups. We excluded *S. anginosus* group isolates that showed group C, G or F antigen in the process of isolate identification.

Surveillance was conducted for 1 year (1 August 2006 to 31 July 2007) in 142 medical institutions participating in the Invasive Streptococcal Disease Working Group established at the 19th Annual Meeting of the Japanese Society for Clinical Microbiology. Invasive streptococcal diseases were defined as conditions with isolation of organisms from a normally sterile site (i.e. blood, cerebrospinal fluid, joint fluid, ascites, or pleural effusion) [1,9,10]. Isolates were first identified as streptococci at local hospital laboratories, using standard commercially available latex agglutination assays. Detailed standardized categories of information regarding clinical features (e.g. hospital departments of initial presentation and underlying conditions) and laboratory findings (e.g. white blood cell (WBC) count, platelet count and C-reactive protein (CRP) serum concentration) were obtained from medical charts for all subjects enrolled. Clinical syndromes were assigned on the basis of physicians' diagnoses recorded in the medical charts. Poor outcomes were defined as either death from invasive infections within 3 weeks of onset or invasive disease-associated sequelae following the streptococcal infection (e.g. worsened paralysis or bedridden status). All isolates were sent to the Laboratory of Molecular Epidemiology for Infectious Agents to determine additional characteristics, including Lancefield group, species, M protein gene (*emm*) or capsular type, and antibiotic susceptibility.

Laboratory methods

Isolates were characterized with standard biochemical and enzymatic tests, and were identified as previously described [11]. The *emm* types of SDSE or GAS isolates [12] and the capsular types of GBS [13] isolates were determined as previously reported. All *emm* typing was based on the CDC database (ftp://ftp.cdc.gov/pub/infectious_diseases/biotech/tsemm/). In addition, we quantified the susceptibility of streptococci to 14 antibiotics, including oral and parenteral antibiotics, by agar plate dilution methods using blood agar, as previously described [10]. Depending on the MICs, the presence of streptococcal genes associated with resistance to antimicrobials (e.g. *mef*(A), *erm*(A), or *erm*(B)) was determined. To assess the similarity of isolates, profiling using pulsed-field gel electrophoresis (PFGE) following DNA treatment with the restriction enzyme *Sma*I was also performed [10].

Statistical analysis

Microsoft Excel was used for data analysis. Patient or pathogen characteristics, clinical features and outcomes were compared between paired groups of isolates (SDSE and GAS, SDSE and GBS, or GAS and GBS), using the chi-squared test. To identify clinical laboratory findings associated with fatal outcome, ORs with 95% CIs and p-values according to the chi-squared test were calculated.

Results

We identified 231 patients with invasive infection caused by SDSE in the records from 142 medical institutions during the 1-year study period, during which time 97 GAS and 151 GBS cases were also collected. All isolates of SDSE, GAS or GBS were referred by the hospital laboratories for further microbiological characterization.

As shown in Fig. 1, the age distribution differed significantly between patients with invasive SDSE and those with GBS infection. All patients (n = 231) with invasive SDSE infection were adults, whereas GBS infected some patients 4 months old or younger in addition to adults, especially the elderly. We therefore chose to compare clinical aspects of invasive SDSE diseases with those caused by GAS (n = 82) or GBS (n = 123) in the adult population (≥15 years old).

Isolates (n = 12) of the *S. anginosus* group were excluded from the current surveillance. Lancefield groups G, C and A, respectively, accounted for 216, 12 and three SDSE isolates.

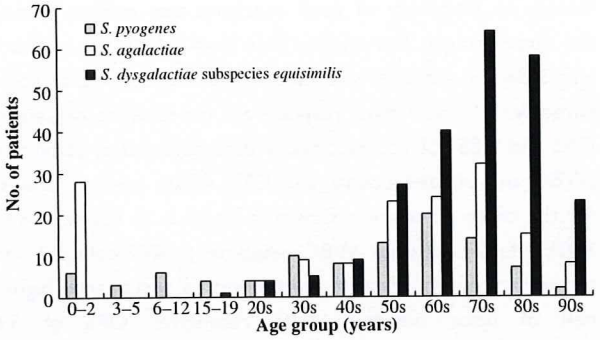

FIG. 1. Number of patients with invasive infections caused by *Streptococcus dysgalactiae* ssp. *equisimilis* (*n* = 231), *Streptococcus pyogenes* (*n* = 97), and *Streptococcus agalactiae* (*n* = 151), shown by age group.

All GAS or GBS isolates were *S. pyogenes* or *S. agalactiae*, respectively.

Patient characteristics and clinical presentations according to the streptococcal group (SDSE, GAS, or GBS)

Patient characteristics, clinical presentations and disease outcomes for all invasive SDSE, GAS and GBS infections are shown in Table 1. The median age of patients with SDSE diseases was 75 years (range, 19–103 years), with the subjects with SDSE infections being significantly older than those with GAS or GBS infections (p <0.01 for each). In our surveillance, all cases of SDSE, GAS and GBS diseases were community-acquired. Forty-two patients (19%) with SDSE infections presented to the hospital emergency department,

TABLE 1. Demographic characteristics, underlying conditions and clinical syndromes in patients with invasive infection caused by *Streptococcus dysgalactiae* ssp. *equisimilis* (*n* = 231), *Streptococcus pyogenes* (*n* = 97), or *Streptococcus agalactiae* (*n* = 151)

Characteristic	No. (%) of patients			p-Value		
	S. dysgalactiae ssp. *equisimilis* (A)	*S. pyogenes* (B)	*S. agalactia* (C)	A vs. B	A vs. C	B vs. C
Demographic characteristic						
Age (years)						
<15	0 (0.0)	15 (15.5)	28 (18.5)	<0.01	<0.01	0.53
15–39	10 (4.3)	18 (22.0)	13 (10.6)	<0.01	0.02	0.03
40–64	50 (21.6)	32 (39.0)	39 (31.7)	<0.01	0.04	0.28
≥65	171 (74.0)	32 (39.0)	71 (57.7)	<0.01	<0.01	<0.01
Gender[a,b]						
Male	117 (51.3)	40 (49.4)	55 (44.7)	0.77	0.24	0.51
Department where presented[a]						
Emergency	42 (19.3)	22 (26.8)	22 (18.0)	0.15	0.78	0.13
Internal medicine	112 (51.4)	36 (43.9)	73 (59.8)	0.25	0.13	0.03
Surgery	64 (29.4)	24 (29.3)	27 (22.1)	0.99	0.15	0.25
Poor outcome[a]						
Death	19 (12.7)	12 (16.7)	8 (10.8)	0.42	0.69	0.30
Death or sequelae	27 (18.0)	19 (26.4)	11 (14.9)	0.15	0.56	0.08
Underlying disease[a]						
None	48 (21.2)	31 (39.7)	16 (11.8)	<0.01	0.07	<0.01
Diabetes mellitus	36 (15.9)	12 (15.4)	22 (16.2)	0.64	0.92	0.72
Malignant disease	35 (15.5)	13 (16.7)	33 (24.3)	0.39	0.02	0.41
Stroke	28 (12.4)	0 (0.0)	5 (3.7)	<0.01	<0.01	0.27
Heart disease	18 (8.0)	4 (5.1)	10 (7.4)	0.80	0.85	0.95
Pulmonary disease	2 (0.9)	0 (0.0)	0 (0.0)	0.93	0.72	–
Liver dysfunction	11 (4.9)	2 (2.6)	17 (12.5)	0.77	<0.01	0.03
Renal dysfunction	13 (5.8)	4 (5.1)	9 (6.6)	0.84	0.72	0.89
Artherosclerotic cardiovascular disease	8 (3.5)	1 (1.3)	11 (8.1)	0.66	0.05	0.12
Autoimmune disease	4 (1.8)	1 (1.3)	5 (3.7)	0.69	0.42	0.68
Other	23 (10.2)	10 (12.8)	8 (5.9)			
Clinical syndrome						
Sepsis without focus	98 (42.4)	27 (32.9)	77 (62.6)	0.06	<0.01	<.01
Cellulitis	52 (22.5)	23 (28.0)	12 (9.8)	0.43	<0.01	<.01
Septic arthritis	23 (10.0)	3 (3.7)	4 (3.3)	0.06	0.02	0.78
Pneumonia	12 (5.2)	6 (7.3)	8 (6.5)	0.74	0.64	0.87
Necrotizing fasciitis	9 (3.9)	5 (6.1)	1 (0.8)	0.67	0.18	0.08
Meningitis	5 (2.2)	3 (3.7)	3 (2.4)	0.79	0.82	0.97
Infectious endocarditis	4 (1.7)	0 (0.0)	4 (3.3)	0.51	0.60	0.24
Streptococcal toxic shock syndorome	2 (0.9)	3 (3.7)	0 (0.0)	0.25	0.76	0.13
Abscess (excluding skin)	2 (0.9)	8 (9.8)	3 (2.4)	<0.01	0.48	0.06
Osteomyelitis	2 (0.9)	0 (0.0)	1 (0.8)	0.94	0.59	0.85
Other	22 (9.5)[c]	4 (4.9)	10 (8.1)			

[a]Patients with unknown data were excluded.
[b]Gender, department where presented, poor outcome, underlying disease and clinical syndrome in the adult population were compared between paired groups (*S. dysgalactiae* ssp. *equisimilis* (SDSE) and group A streptococcus (GAS), SDSE and group B streptococcus (GBS), or GAS and GBS).
[c]Others included urosepsis (*n* = 13), septic spondylitis (*n* = 4), endophthalmitis (*n* = 2), peritonitis (*n* = 2), and biliary tract infection (*n* = 1).

a fraction similar to those of the subjects presenting to the emergency department with infections involving the other two groups.

Underlying diseases were present in 79% of patients with invasive SDSE illnesses; underlying medical conditions in subjects with GAS infections were significantly less frequent than in patients with SDSE or GBS infections ($p < 0.01$ for each). Among patients with SDSE infection, stroke was significantly more frequent than in patients with GAS or GBS infection ($p < 0.01$ for each), whereas in patients with GBS infection, liver dysfunction was significantly more prevalent than in those with SDSE or GAS infection ($p < 0.01$ and $p = 0.03$, respectively). With regard to clinical syndromes, sepsis without focus was significantly more frequent among GBS-infected than among SDSE-infected or GAS-infected patients ($p < 0.01$ for each), whereas cellulitis was significantly less frequent among GBS-infected patients than among SDSE-infected or GAS-infected patients ($p < 0.01$ for each). Subjects infected with SDSE presented more often with septic arthritis than those infected with GBS ($p = 0.02$), whereas patients with GAS infections more often had abscesses involving sites deeper than the skin than did patients with SDSE infection ($p < 0.01$).

Disease outcomes according to the streptococcal group (SDSE, GAS, or GBS)

Among 150 patients (65%) with invasive SDSE diseases for whom follow-up investigation was complete, 19 (13%) died, and eight (5%) had post-infection sequelae. No significant difference in frequency of poor outcome was evident among the three groups. The median time from admission to death was 3 days in subjects who died of SDSE infections, as compared with 1 and 7 days, respectively, for fatalities caused by GAS and GBS. Clinical laboratory data obtained at admission (WBC and platelet counts and CRP serum concentrations) for the three groups are shown in Table 2. In subjects with SDSE infection, a poor WBC response (<5000 cells/μL) and thrombocytopenia were associated with a significantly higher risk of poor outcome, with respective ORs of 3.6 (95% CI 1.2–11.5; $p = 0.04$) and 4.5 (95% CI 1.6–12.2; $p < 0.01$). These two findings were also significantly related to poor outcomes in patients with GAS infections, but not in adults with GBS infections. There was no association of CRP level with poor outcome in the three groups.

emm typing for the isolates from adults

emm typing was carried out for 231 SDSE isolates and for 82 of the 97 *S. pyogenes* isolates; capsular typing was performed for 123 of the 151 *S. agalactiae* isolates. The incidences of *emm* types among SDSE isolates are shown in Table 3. Interestingly, *emm* type stG6792 in SDSE isolates, which was confirmed most frequently ($n = 55$, 24%), was significantly associated with poor outcome of invasive SDSE diseases in comparison with other SDSE *emm* types, with an OR of 2.4 (95% CI 1.0–5.9; p 0.04). Within the stG6792 type, most isolates showed *emm* type stG6792.3 ($n = 54$), whereas PFGE of the isolates of the stG6792.3 type revealed similar profiles. On the other hand, among GAS isolates, *emm* type 1

TABLE 2. Clinical laboratory findings associated with fatal outcome of invasive infections caused by *Streptococcus dysgalactiae* ssp. *equisimilis*, *Streptococcus pyogenes*, or *Streptococcus agalactiae*

	Median or percentage (25/75th percentiles) and (no./total)			
	Poor outcome[a]	Without poor outcome[a]	Analytical data (OR (95% CI))	p-Value
S. dysgalactiae ssp. *equisimilis*				
WBC (/μL)	11 400 (3908–15 200) (22/27)	12 600 (7850–16 050) (107/123)	3.6 (1.2–11.5)	0.04
<5000/μL	27.3% (6/22)	9.3% (10/107)		
PLT (10^4/μL)	11.1 (7.3–15.2) (21/27)	18.8 (12.3–24.5) (100/123)	4.5 (1.6–12.2)	<0.01
<13.0 × 10^4/μL	66.7% (14/21)	13.0% (31/100)		
C-reactive protein (mg/dL)	18.7 (12.3–26.8) (22/27)	4.9 (1.6–21.1) (103/123)	0.2 (0.03–1.8)	0.23
<1 mg/dL	4.5% (1/22)	17.5% (18/103)		
S. pyogenes				
WBC (/μL)	7200 (3800–19 075) (18/19)	10 300 (8000–15 015) (46/53)	4.2 (1.2–15.6)	0.04
<5000/μL	38.9% (7/18)	13.0% (6/46)		
PLT (10^4/μL)	10.1 (7.4–16.5) (18/19)	19.4 (15.6–28.0) (46/53)	7.5 (2.2–25.8)	<0.01
<13.0 × 10^4/μL	61.1% (11/18)	17.4% (8/46)		
C-reactive protein (mg/dL)	22.1 (12.7–28.0) (17/19)	15.6 (5.4–24.6) (45/53)	0.6 (0.06–6.5)	0.89
<1 mg/dL	5.9% (1/17)	8.9% (4/45)		
S. agalactiae				
WBC (/μL)	14 350 (9200–17 225) (10/11)	11 600 (7800–15 150) (47/63)	1.2 (0.1–12.6)	0.64
<5000/μL	10.0% (1/10)	8.5% (4/47)		
PLT (10^4/μL)	10.0 (7.7–18.9) (9/11)	17.9 (12.2–24.3) (46/63)	3.2 (0.7–14.1)	0.23
<13.0 × 10^4/μL	55.6% (5/9)	28.3% (13/46)		
C-reactive protein (mg/dL)	6.7 (1.6–10.0) (9/11)	8.3 (1.1–19.0) (46/63)	1.2 (0.2–6.9)	0.78
<1 mg/dL	22.2% (2/9)	19.6% (9/46)		

WBC, white blood cell count; PLT, platelet count.
[a]Patients with unknown data were excluded.

TABLE 3. Types of *emm* for 231 cases of invasive infection caused by *Streptococcus dysgalactiae* ssp. *equisimilis*

emm type	No. of isolates (n = 231)	Poor outcome (n = 27)
stG6792[a]	55 (23.8)	11
stG485	37 (16.0)	1
stG6	21 (9.1)	4
stG10	21 (9.1)	2
stG652	17 (7.4)	1
stG2078	16 (6.9)	2
stC36	15 (6.5)	1
stG245	13 (5.6)	2
stG5420	6 (2.6)	1
stG480	5 (2.2)	1
stC6979	5 (2.2)	0
stG4974	4 (1.7)	1
stG653	3 (1.3)	0
Other	11 (4.8)	0
Non-typeable	2 (0.9)	0

[a]This *emm* type includes stG6792.3 (n = 54) and stG6792.0 (n = 1).

was found most frequently (n = 27, 33%). This type was also significantly related to poor outcome of invasive infections, with an OR of 3.4 (95% CI 1.1–10.5; p 0.02). Other *emm* types frequently found in GAS infections were 49 (n = 12), 89 (n = 5), 11 (n = 4), 12 (n = 4), and 75 (n = 4), whereas *emm* types 12 and 6 predominated in our study of non-invasive GAS infections (T. Wajima, S. Y. Murayama & K. Ubukata, unpublished data).

Capsular type Ib in GBS isolates were not associated with poor outcome of invasive diseases. This type (n = 39) was observed most frequently in adults. Other GBS capsular types, i.e. V (n = 23), II (n = 15), III (n = 14), and Ia (n = 11), were predominantly observed, showing distribution patterns different from those found in the study of non-invasive GBS infections.

Antibiotic susceptibility

Antibiotic susceptibility testing was performed for all isolates of SDSE, GAS and GBS. Of 231 SDSE isolates, four had the *mef*(A) gene, and 13 and six isolates had *erm*(A) and *erm*(B) genes, respectively; the presence of the latter suggested a high level of resistance to clarithromycin (MIC ≥64 mg/L). In addition, two SDSE isolates showed resistance to fluoroquinolones, such as levofloxacin (MIC ≥32 mg/L), based on substitutions in GyrA (Ser81 to Phe or Tyr) and ParC (Ser79 to Tyr). No penicillin or cephalosporin resistance was observed.

Discussion

To the best of our knowledge, this is the first nationwide surveillance regarding invasive infections caused by SDSE in Japan. The study demonstrates significant differences in clinical aspects, including prognosis, between disease entities caused by SDSE, GAS and GBS. The mortality rate of invasive SDSE diseases in our study (13%) was similar to those previously described in other countries (8–18%) [1,5,14,15].

Host-protective factors, including WBCs, platelets, and CRP, affect the severity and prognosis of infectious diseases, especially those involving invasive pathogens. Disturbed haemostasis is a central finding in severe *S. pyogenes* infection that is associated with M protein-induced platelet activation and thrombus formation [16]. In particular, microthrombi are found both at the local site of infection and at distant sites. Platelets are responsible for maintaining vascular function and haemostasis. With regard to WBCs and CRP, streptococcal erythrogenic toxin B induces apoptosis and proliferation in human leukocytes [17]. Moreover, serial CRP monitoring was found to alert physicians to complications and predict outcome earlier than clinical signs or roentgenograms in 63 children with acute haematogenous osteomyelitis [18]. In our investigations, a poor WBC response (<5000 cells/μL) and thrombocytopenia at admission each showed a significantly higher risk of poor outcome (death or invasive disease-associated sequelae) in patients with invasive SDSE or GAS infections. However, CRP could not predict poor outcomes. We need to continue to examine associations between prognosis and host defence factors.

The M protein of *S. pyogenes* is a major bacterial virulence factor that confers resistance to phagocytosis [19]. Recently, analysis of the *emm* gene, which codes for the amino acid sequence (variable region) at the N-terminal end of the M protein, has often been used for molecular epidemiological studies regarding outbreaks of invasive or non-invasive streptococcal infections [20,21]. Data suggesting horizontal gene transfer and recombination between the *emm* genes of SDSE and GAS strains have been observed in clinical isolates from seven subjects [21]. These genetic transfer and recombination events might have a role in pathogenesis. In our study, *emm* type stG6792 in SDSE isolates (n = 55), the type most frequently confirmed in SDSE infection, was significantly more often associated with poor outcome of invasive diseases than other SDSE *emm* types. In contrast to the high frequency in our study, only three strains of stG6792 were observed in a recent article from the USA [1]. Surveillance periods for strains differed between Japan (2006–2007) and the USA (2002–2004). On the basis of the CDC database concerning *emm* type sequences, the stG6792.3 reference strain appeared to be derived from a streptococcal isolate from India, suggesting that this strain might have spread from India to Japan. Similarly, the *emm1* type in GAS isolates (n = 27), the type that we found most frequently, was significantly related to poor outcome of invasive GAS infections.

Relationships between *emm* types and prognosis of the SDSE and GAS infections in Japan should be investigated in an ongoing manner. In addition, we need to determine person-to-person transmission routes of SDSE, as the stG6792.3 strain (n = 54) was observed most frequently within the stG6792 type, and similar *Sma*I digestion patterns were found for the isolates using PFGE analysis.

Antibiotic susceptibility testing and resistance gene identification for SDSE isolates in our study revealed clarithromycin and levofloxacin resistance, as described by others [1,2,5]. On the other hand, susceptibility to β-lactam antibiotics, including penicillin and cephalosporins, was high, suggesting that they should be the usual drugs of choice.

Some limitations of our investigation should be noted. Broyles *et al.* [1] recently reported an annual incidence of invasive β-haemolytic streptococcal infections involving groups other than A and B in the USA of 3.17 cases per 100 000 persons. Our surveillance, on the other hand, was not a population-based study of the burden of infections caused by SDSE, GAS and GBS, as official, systemic surveillance has not yet been established in Japan. To our knowledge, however, our study is the largest record of cases of invasive illness caused by SDSE, GAS and GBS in Japan to date.

In our investigation, clinical aspects of invasive SDSE infections appear to differ from those caused by GBS, and to be somewhat more similar to those caused by GAS. These observations might be accounted for by our findings that genes encoding virulence factors (e.g. M protein) in SDSE could be partially shared with those in GAS, on the basis of results of whole genome analyses of original SDSE isolates. Moreover, clinical isolates of SDSE possessing group A antigen have been reported [7]. SDSE has been established as a possible component of the normal flora of the skin, oropharynx, and gastrointestinal and genitourinary tracts. We identified SDSE in respiratory tract specimens from patients with non-invasive SDSE diseases in another study (K. Sunaoshi, S. Y. Murayama & K. Ubukata, unpublished data). However, questions persist as to how SDSE invades deep tissues and vessels. Further investigations to clarify this issue are needed.

In conclusion, primary-care doctors, particularly those treating patients in emergency departments, should consider invasive diseases caused by SDSE, especially when treating elderly subjects with underlying medical conditions.

Acknowledgements

This study, aiming to clarify clinical aspects in invasive streptococcal infections and to determine the molecular epidemiology, was presented in part at a workshop at the 19th Annual Meeting of the Japanese Society for Clinical Microbiology in January 2008 (Edogawa-ku, Tokyo, Japan). We thank A. Ono, N. Chiba and K. Okada for assistance with manuscript preparation. We also express our thanks to staff members at all participating institutions.

Transparency Declaration

This work was supported in part by a grant under the category, 'Research Project for Emerging and Re-emerging Infectious Diseases' (H-19-002), from the Ministry of Health, Labour and Welfare of Japan (to K. Ubukata). None of the authors is aware of any relationship or any degree of conflicting or dual interest, financial or of any other nature, that may affect professional judgement in relation to the article.

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